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PhD Thesis

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Structural and functional brain signatures of sex-hormone transitions and implications for perinatal mental health

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Frontpage illustration by Marlene Gørtz Jønsson

Dedicated to Michael

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Summary (English)

Across pregnancy and the postpartum period (i.e., the perinatal period), women often experience mental distress symptoms and about 10-15% of pregnant and postpartum women develop a depression. The dramatic sex-hormone reductions from late in pregnancy (antepartum) to the early postpartum period have been proposed as a possible risk mechanism. Currently there is little consensus on their role, but estrogen sensitivity, at a genomic level, may be a risk marker for depression. Some studies show that serotonergic signaling may be involved depressive responses during sex-hormone transitions. Further, recent data show that sex-hormone transitions, including the peripartum are associated with changes in hippocampal plasticity.

The aim of this thesis was to determine the associations between mental distress, the estrogen estradiol, markers of serotonergic signaling and hippocampal volume in relation to pharmacological and natural models of sex-hormone transitions in healthy women.

In Study 1, we evaluated if pharmacologically induced estradiol changes were associated with hippocampal volume changes. In a randomized, placebo-controlled study design (N = 60), we previously showed that a gonadotrophin-releasing hormone agonist (GnRHa) induced subclinical depressive symptoms, compared to placebo, in a manner that depended on the magnitude of the GnRHa-induced estradiol decrease and brain serotonin transporter (SERT) binding. Here, we show that hippocampal volume changes were similar in response to GnRHa and to placebo, but that GnRHa-induced estradiol decreases were associated with hippocampal volume reductions, adjusted for SERT contributions. This did not map on to GnRHa-induced depressive symptoms. Thus, our data support that estradiol may be associated with hippocampal volume changes, but not in a depressogenic way.

In Study 2, we followed 100 healthy pregnant women from late in pregnancy to week five postpartum (82 completed follow-up). We only included women planned to deliver by caesarean section (C-section), as this allowed us to collect cerebrospinal fluid (CSF) during spinal anesthesia. We found that high antepartum CSF levels of the main serotonin metabolite, 5-HIAA, was associated with more mental distress symptoms at week five. However, a large decrease in estradiol, from late in pregnancy to week five postpartum, was associated with *less* mental distress symptoms. Under the assumption that 5-HIAA is a marker for pregnancy induced SERT expression, we speculate that increased brain SERT levels may carry over the postpartum period

and convey susceptibility to mental distress. Further, that healthy women may benefit from large estradiol changes, but that such protective mechanisms may disrupted in women who develop a depression, perhaps linked to estrogen sensitivity.

In Study 3, a subgroup of the healthy pregnant women was recruited for a neuroimaging program (N = 31). This included quantification of cerebral of 5-HT4R with positron emission tomography (PET, n = 23) and quantification of hippocampal volume with magnetic resonance imaging (MR, n = 31), five weeks postpartum. Data from 24 female controls, available through the Center for Integrated Molecular Brain Imaging (CIMBI) database, were also included.

Postpartum women and healthy controls had similar 5-HT4R binding and hippocampal volume. Within the postpartum group, subclinical depressive symptoms were associated with a higher hippocampal volume, but not with 5-HT4R. Further, high cerebral 5-HT4R in interaction with a large decrease in estradiol was associated with a smaller hippocampal volume. We speculate that high hippocampal volume reflects a maladaptive hippocampal plasticity. Further, 5-HT4R in interaction with peripartum estradiol changes seems to play a key role in postpartum hippocampal plasticity. This may have relevance in relation to depressive responses during sex-hormone transitions.

Taken together, our results indicate that in healthy women, the brain adapts well to sex-hormone transitions. Further, that both estradiol and serotonergic signaling may be important for hippocampal plasticity in relation to sex-hormone transitions, but that the length and magnitude of an estradiol fluctuation, may affect how it maps on to hippocampal volume and subclinical depressive symptoms. Our data points towards potential risk mechanisms for depressive responses during sex-hormone transitions, however further evaluation in clinical and high risk samples is required.

Resume (dansk)

Mange kvinder oplever humør, søvn og angstproblemer i graviditeten og efter fødslen (postpartum), og omkring 10-15 % udvikler en perinatal depression, dvs. depression i graviditeten eller postpartum. Dette kan hænge sammen de store kønshormonelle ændringer fra sent i graviditeten til den tidlige postpartum-periode, men der er ikke konsensus på området. Nyere studier peger i stedet på at østrogenfølsomhed på genomisk niveau som en mulig risikomarkør. Nogle studier tyder på at hjernens serotonerge system spiller on rolle for udvikling af depressive symptomer i relation til kønshormonelle ændringer. Ydermere viser nyere studier at plasticiteten i hippocampus er ændret i forbindelse med kønshormonelle overgangsfaser, inklusiv den perinatale periode. Formålet med denne afhandling var at bestemme sammenhængen mellem mentalt velbefidende, kønshormonet østradiol, serotonerge markører, og volumen af hippocampus i relation til kønshormonelle manipulationer i raske kvinder og de naturlig kønshormonelle ændringer, der sker i forbindelse med graviditet og fødsel.

I Studie 1 undersøgte vi, om farmakologisk inducerede østradiolændringer var associeret med ændringer i hippocampus volumen. I et randomiseret, placebokontrolleret studie (N = 60) har vi tidligere vist, at en syntetisk analog til "gonadotropin releasing hormone" (GnRHa) kan fremprovokere subkliniske depressive symptomer, sammenlignet med placebo. Fremkomsten af depressive symptomer afhang af størrelsen på det GnRHa-inducerede fald i kønshormonet østradiol, samt serotonintransporter (SERT) bindingen i hjernen. I Studie 1 fandt vi ingen forskel i hippocampus-volumenændringer mellem kvinder, der fik GnRHa, og kvinder, der fik placebo. I GnRHa-gruppen var mindsket hippocampusvolumen imidlertid associeret ned størrelsen på østradiolfaldet, justeret for SERT binding ved baseline. Der var ingen association mellem ændring i hippocampusvolumen og depressive symptomer. Vores data derfor peger på at de GnRHa-inducerede østradiolændringer var koblet til ændringer i hippocampus, men ikke både en måde der førte til depressive symptomer.

I studie 2 fulgte vi 100 raske gravide kvinder fra sidst i graviditeten til uge fem postpartum. Vi inkluderede kun kvinder, der skulle føde ved planlagt kejsersnit, for at kunne indsamle cerebrospinalvæske (CSV) i forbindelse med deres rygmarvsbedøvelse (82 kvinder gennemførte

studiet). Høje niveauer af serotoninmetabolitten, 5-HIAA, i CSV var forbundet med subkliniske depressive symptomer fem uger efter fødslen. Desuden var et stort østradiolfald, fra sidst i graviditeten til uge fem efter fødslen, forbundet med øget mentalt velbefindende i uge 5 postpartum. Under antagelse af at 5-HIAA er en markør for hjernen SERT niveauer i graviditeten, kan dette pege på at høje SERT niveauer i graviditeten bringes med over i postpartum perioden og subkliniske depressive symptomer. Desuden ser et stort østrogenfald ud til at vær gavnlig for raske kvinder. Disse gavnlige mekanismer er formentlig sat ud af funktion i kvinder med depression, måske på baggrund af genomisk østrogenfølsomhed.

I studie 3 blev en undergruppe af de raske gravide kvinder rekrutteret til et hjerneskanningsprogram (N = 31). Dette inkluderede *in vivo* molekylær billeddannelse af 5-HT4R med positron-emissions-tomografi (PET, n = 23) og strukturelle magnetisk resonans skanninger (MR, n = 31), fem uger efter fødslen. Data fra 24 kvindelige raske kontroller blev inkluderet fra Center for Integrated Molecular Brain Imaging (CIMBI) databasen. Postpartum kvinder og raske kontroller havde ikke signifikante forskelle i 5-HT4R binding i hjernen eller hippocampus volumen. I postpartum-gruppen var subkliniske depressive symptomer associeret med et højere hippocampusvolumen, men der var ingen association med 5-HT4R. Imidlertid var der en interaktion imellem 5-HT4R i hjernen og østradiolfaldet på hippocampus volumen, således at et større østradiolfald og en højere 5-HT4R binding var associeret med et mindre hippocampusvolumen. Vi mistænker at et højt hippocampus volumen kan afspejle perinatal maladaptation i hippocampus plasticiteten. Desuden peger vores data på at 5-HT4R og østradiolændringer tilsammen kan have betydning for hippocampusplasticiteten. Dette er muligvis relevant i forbindelse med udvikling af depression i forbindelse med kønshormonelle overgangsfaser.

Tilsammen peger vores resultater i retning af at hjernen tilpasser sig kønshormonelle ændringer godt i raske kvinder. Både det serotonerge system og østradiolændringer har betydning for hippocampusplasitciteten, dog afhænger østradiols virkninger af længden og størrelsen på østradiolfluktuationerne.

List of manuscripts

The thesis is based on the following manuscripts:

Manuscript 1:

Camilla Borgsted, Emma Hoegsted, Susanne Henningson, Anja Pinborg, Melanie Ganz and Vibe G. Frokjaer. Hippocampal volume brain changes in a pharmacological sex-hormone manipulation model for depression in women. *Hormones and Behavior (in revision). Revised edition.*

Manuscript 2:

Camilla Borgsted, Stinne Høgh, Emma Sofie Høgsted, Laura Fonnesbech-Sandberg, Kim Ekelund, Charlotte Krebs Albrechtsen, Julie Therese Wiis, Hanne Hegaard, Eleonora Cvetanovska, Anders Juul, Hanne Frederiksen, Anja Pinborg, Pia Weikop, and Vibe Frokjaer. The role of central serotonergic markers and estradiol changes in perinatal mental health. *In final prep.*
At subset of the data from this paper has formed the basis of Laura Fonnesbech-Sandberg's Master's thesis.

Manuscript 3:

Camilla Borgsted, Emma Sofie Høgsted, Stinne Høgh, Eleonora Cvetanovska, Kim Ekelund, Charlotte Albrechtsen, Julie Wiis, Anja Pinborg, Hanne Hegaard, Hanne Frederiksen, Anders Juul, Pia Weikop, Gitte Moos Knudsen, Claus Svarer, Melanie Ganz and Vibe G. Frokjaer. Serotonin 4 receptor and hippocampal signatures of healthy peripartum brain adaptations. *In prep*

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Abbreviations

GnRHa	Gonadotrophine-releasing hormone agonist
5-HIAA	5-hydroxyindolacetic acid
5-HT	Serotonin
5-HT4R	Serotonin 4 receptor
5-HTTLPR	Serotonin Transporter-linked Promotor Region
AIC	Akaike information criterion
BMI	Body Mass Index
BPND	Non-displaceable binding potential
cAMP	Cyclic adenosine monophosphate
CI	Confidence Interval
CIMBI	Center for Integrated Molecular Brain Imaging
COVID-19	Coronavrus Disease 2019
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic Acid
DSM-IV	Diagnostic Statistics Manual, version 4
DSM-V	Diagnostic Statistics Manual, version 5
EPDS	Edinburgh Postnatal Depression Scale
HAMD-17	Hamilton depression rating scale, 17-item
HVA	Homovanillic acid
ICD-10	International Statistical Classification of Diseases 10th revision
LC-MS/MS	liquid chromatography tandem mass spectrometry
MAO	Monoamine oxidase
MAO-A	Monoamine oxidase A
MBq	Mega becquerel (number of radioactive decays per second)
MDI	Major Depression Inventory
MOPEG	3-methoxy-4-hydroxyphenylglycol
MPRAGE	Magnetization prepared rapid gradient echo
MR	Magnetic Resonance Imaging

MRI	Magnetic Resonance Imaging
NE	Norepinephrine
PCR	Polymerase chain reaction
PET	Positron Emission Tomography
PND	Perinatal depression
PSQI	Pittsburgh Sleep Quality Inventory
RF	Radiofrequency
SD	Standard Deviation
SERT	Serotonin transporter
SRTM	Simplified reference tissue model
SSRI	Selective serotonin reuptake inhibitors
STAI	Stata-Trait Anxiety Inventory
TAC	Time-Activity Curve
TE	Echo time
TI	Inversion time
TR	Repetition time
VOI	volume of interest

Background

Perinatal mental distress and depression

The perinatal period covers pregnancy and the postpartum period and is often associated with increased levels mental distress. Both anxiety symptoms and sleep disturbances are common (30-40% and ~40%, respectively), and at least 50% of women experience "postpartum blues", i.e., transiently low mood and susceptibility to crying, that lasts a few days or weeks (Beck, 2001; Blackmore et al., 2016; Emamian et al., 2019; Grant et al., 2008; Harris et al., 1994; O'Hara et al., 1991; Richter et al., 2019; Robertson et al., 2004; Salari et al., 2021). The reported proportion in these studies vary, but about 30-40% of pregnant and postpartum women have anxiety symptoms, at least 40% have perinatal sleep disturbances and around 50% develop blues symptoms. Importantly, these common and subclinical levels of distress increase the risk of developing manifest depressive episodes (Emamian et al., 2019; Reck et al., 2009; Robertson et al., 2004). DSM-V defines perinatal depression (PND) as a depressive episode with onset during pregnancy or up to four weeks postpartum (American Psychiatric Association. DSM-5 Task Force., 2013). Depressive symptoms in relation to pregnancy and birth are common, as around 10-15% of new mothers develop a depressive episode (Gavin et al., 2005; Howard et al., 2014; Meltzer-Brody et al., 2013). PND is characterized by a high degree of anxiety and anhedonia symptoms, but there are also reports of cognitive problems (Hampson et al., 2015; Putnam et al., 2017). PND may have grave consequences for both mother and child; in particular, suicide constitutes a prominent cause for maternal death in developed countries (Bødker et al., 2021b, 2021a). Furthermore, maternal depression has been shown to affect the cognitive development and future mental health of the infant (Evans et al., 2012; Glover, 2014; Goodman et al., 2011; Stein et al., 2014). Previous studies have identified a number of risk factors for the development of PND such as adverse life events, a history of depression, previous PND, high or low maternal age and primiparity, but the underlying mechanisms remain unclear (Aasheim et al., 2012; García-Blanco et al., 2017; Gavin et al., 2005; Guintivano et al., 2018; Meltzer-Brody et al., 2017, 2013; Munk-Olsen et al., 2014; Robertson et al., 2004; Van Niel and Payne, 2020; Wisner et al., 2004). The risk for a severe depressive episode is highest around 4-8 weeks postpartum (Putnam et al., 2017). In line

with this, the risk for hospital admission for any psychiatric disorder, indicating severe mental illness, is also increased in new mothers in this early postpartum period with a peak as early as 10-19 days postpartum (Munk-Olsen et al., 2006). Notably, the same study found that risk for hospital admission was lower in new fathers, and there was no early postpartum peak. This indicates the psychological stress in relation to childbirth and parenthood not alone can explain the early postpartum peak in severe mental illness that women experience. Thus, it is likely that biological factors play a role. Combined, these studies points towards a window of vulnerability in the first months postpartum, which may map on to biological factors rather than psychological stress.

Sex-hormone transitions and mental distress

The lifetime risk for depression is almost twice as high in women compared to men. The reason for this remains unknown, but fluctuations in sex-hormones likely play a role as the risk for a depression is particularly high for women during hormone transitions phases such as puberty, pregnancy and birth, and perimenopause, and in relation to use of hormonal contraceptives (Kuehner, 2017; Skovlund et al., 2016; Soares and Zitek, 2008). It has been suggested that the emergence of depressive symptoms in relation to sex-hormone transitions may be associated with the magnitude of the sex-hormone changes (Bloch et al., 2000; Frokjaer et al., 2015; Soares and Zitek, 2008). Three major estrogens are important during different life phases: Estrone (E1) plays a key role after menopause and to some extent also postpartum; estradiol (E2) is by far the most important during reproductive years; and estriol (E3) is particularly abundant during pregnancy. The early postpartum window is characterized by dramatic hormone fluctuations, especially for estradiol and estriol. They both increase steadily to very high levels during pregnancy (more than x300 compared to naturally cycling women) and are abruptly reduced to hypogonadal levels after delivery and expulsion of the steroid producing placenta, see **Figure 1** for a schematic illustration (de Rezende et al., 2019; Kuijper et al., 2013; Qiu et al., 2020; wilson et al., 1980). In breastfeeding women, they may remain low for months (McNeilly et al., 1982). Thus, the period that is associated with the highest risk of a severe perinatal depression is also characterized by the most dramatic estrogen changes.

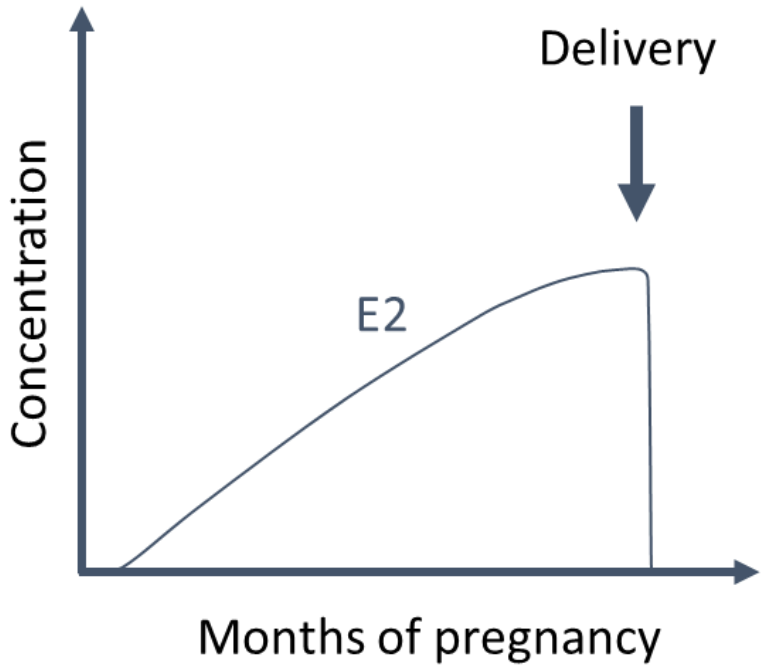


Figure 1. Schematic illustration of estradiol (E2) concentrations across pregnancy.

However, so far most studies do not find a link between perinatal depression and absolute levels of estrogens or perinatal changes in estrogens (Chatzicharalampous et al., 2011; Mehta et al., 2014; Okun et al., 2011; T. Abou-Saleh et al., 1998). Some studies have reported a negative association between estradiol levels and depressive symptoms in pregnancy and postpartum (Fan et al., 2009; O'Hara et al., 1991). A few studies have found that higher estrogen postpartum was associated with early postpartum mental distress or depressive symptoms (Heidrich et al., 1994; Klier et al., 2007). However, firm conclusions are difficult to draw as the studies differ in sample size, degree of clinically relevant symptoms, absolute estradiol levels versus changes, sensitivity of the quantification method and when in the perinatal period the blood samples used to determine hormone levels were drawn. Relatively few studies have evaluated changes in estradiol, although research done in relation to other sex-hormone transitions suggest that this may be important in the emergence of depressive symptoms (Bloch et al., 2000; Freeman et al., 2006; Frokjaer et al., 2015). However, recent studies also point towards an altered sensitivity to sex-hormones in the women who develop PND. One study showed that women with previous PND developed depressive symptoms in response to a sex-hormone manipulation that simulated perinatal estradiol and progesterone fluctuations, although over a shorter timeframe (Bloch et al., 2000).

Similarly, recent studies implicate genomic estrogen sensitivity markers, i.e., DNA methylation and gene-transcription profiles, in risk for depression (Bloch et al., 2000; Guintivano et al., 2014; Mehta et al., 2019, 2014; Osborne et al., 2016). Intriguingly, in a pharmacological sex-hormone manipulation risk model, conceptualized by our group, these genomic risk markers mapped on to depressive symptoms, estradiol changes, and changes in the cerebral serotonin system, see **Figure 2** for study design (Frokjaer, 2020; Frokjaer et al., 2015; Mehta et al., 2019). In this model, healthy women with regular menstrual cycles received either a placebo or gonadotrophin releasing hormone (GnRHa), which first stimulates and then completely downregulates endogenous sex-hormones. Compared to placebo, GnRHa intervention induced a higher level of subclinical depressive symptoms in a manner dependent on the magnitude of the decrease in estradiol from before intervention to the completely downregulated state after intervention. This was associated with heightened serotonin transporter brain binding, which promoted depressive symptoms in interaction with changes in estradiol, but also altered hippocampal resting-state functional connectivity, increased reactivity to emotional stimuli in insula, and reduced amygdala response to reward (Fisher et al., 2017; Frokjaer et al., 2015; Henningsson et al., 2015; Macoveanu et al., 2016). Although the window of vulnerability seems to overlap with dramatic changes in endogenous estrogens, there is conflicting evidence of their direct role in perinatal mental distress. However, factors like estrogen sensitivity may play a role.

Baseline	**Intervention**	**Bleeding**	**Follow-up (1) Stimulation phase**	**Follow-up (2) Supression phase**	**Exit**
CD 6.6 ± 2.2	*CD 22*	*9 ± 3 days p.i.*	*4 ± 2 days p.i.*	*16.2 ± 2.6 days p.i.*	*30 days p.i*
Hamilton score Brain imaging Neuropsychology Gene expression	GnRHa (*n* = 30) Placebo (*n* = 30)	GnRHa: 10 ± 3 days p.i Placebo: 7 ± 3 days p.i	Subgroup of n = 38 Gene expression	Hamilton score Imaging Neuropsychology Gene expression	*SE*

Abbreviations: CD, cycle day; p.i, post intervention.
SE: Side effects score day 7, 12 and 30 p.i.

Figure 2. Overview of the study design for a pharmacological sex-hormone manipulation risk model for depression. The figure is reproduced from (Frokjaer, 2020), with permission from the author and the publishers.

The serotonin system and sex-hormone transitions

Sex-hormones shape the adult female brain through hormone transitions phases, including major neurotransmitter systems, such as the serotonin (5-HT) signaling system (Barth et al., 2015). Especially estrogens, such as estradiol, potently affect key features of the 5-HT system (Bethea et al., 2002; Borrow and Cameron, 2014; Deecher et al., 2008; Lu et al., 2003; Suda et al., 2008; Sumner et al., 2007). Although relatively scarce, animal studies show that the 5-HT system may function substantially different in relation to pregnancy and birth and may be key in healthy maternal behaviors (Lonstein, 2019; Pawluski et al., 2019). Furthermore, human genetic and *in vivo* molecular imaging studies indicate the serotonergic signaling may play a key role in mental well-being during the perinatal period. One study found that brain levels of the inhibitory autoreceptor 5-HT 1 receptor (5-HT1R) in women with postpartum depression was reduced compared to non-depressed postpartum controls (Moses-Kolko et al., 2008). Animal data indicate that 5-HT1R is reduced in response to exogenous estrogens and may play a role in maternal behaviors, mainly maternal aggression, and response to stress in the perinatal period (Bethea et al., 2002; Lokuge et al., 2011; Pawluski et al., 2019). Estrogens also regulate monoamine oxidase A (MAO-A) metabolism of monoaminergic neurotransmitters, including 5-HT; increased levels of estrogens are associated with decreased MAO-A activity (Bethea et al., 2002; Deecher et al., 2008). Intriguingly, the MAO-A level is elevated for the first few days postpartum, a period characterized by transient mental distress known as postpartum blues (Sacher et al., 2010). Further, MAO-A levels remain elevated in women who develop postpartum depression and subclinical levels of mental distress (Sacher et al., 2015). In addition, although the results are not entirely consistent, current evidence suggests that long-term estradiol exposure may induce expression of the main synaptic regulator of 5-HT: The serotonin transporter (SERT; Deecher et al., 2008; Lu et al., 2003; Sumner et al., 2007). SERT gene expression is regulated by a polymorphism in the promotor region of the SERT gene (5-HTTLPR; Heils et al., 1996; Praschak-Rieder et al., 2007). Women who carry the high-expressing variant (long allele) have an increased risk for PND in a “gene-dose” dependent manner, meaning that women who carry two copies are at higher risk (Sanjuán et al., 2008; Tavares Pinheiro et al., 2013). In line with this, data from our pharmacological sex-hormone manipulation risk model for depression, showed that within the healthy women who received GnRHa, there was an interaction between change in brain SERT and

the magnitude of the decrease in estradiol on the emergence of subclinical depressive symptoms, such that the association between depressive symptoms and change in estradiol was more pronounced in women with an increase SERT binding, compared to women with a decrease in SERT binding. Compared to women with a small change in brain SERT, the association between change in estradiol and the emergence of depressive symptoms was more pronounced in women with heightened SERT (Frokjaer et al., 2015). Further, genomic data (DNA methylation and gene-transcription profiles) supported that the changes in brain SERT availability and markers for estrogen sensitivity were associated (Mehta et al., 2019).

Intriguingly, animal data and human genetic and pharmacological studies suggests that long-term increased brain SERT levels may be associated with increased cerebrospinal fluid (CSF) levels of the main serotonin metabolite, 5-hydroxyindolacetic acid (5-HIAA; Bartolomucci et al., 2010; Carpenter et al., 2003; De Bellis et al., 1993; Cecilie Löe Licht et al., 2010; Mårtensson et al., 1991; Nikisch et al., 2004; Potter et al., 1985; Williams et al., 2003; Y et al., 1997). 5-HIAA is the result of MAO-A degradation of 5-HT and is considered a marker of serotonergic turnover (Bethea et al., 2002). Both MAO-A and SERT are targets for common antidepressants (Pereira and Hiroaki-Sato, 2018) and studies suggests that a reduction in 5-HIAA is associated with antidepressant drug treatment (De Bellis et al., 1993; Mårtensson et al., 1991; Nikisch et al., 2004; Potter et al., 1985; Sheline et al., 1997). Thus, by measuring 5-HIAA, it may provide insight into some of the risk mechanisms that may be at work in pregnancy, without a need for molecular imaging methods which are contraindicated in pregnancy due to radiation exposure. Yet, it is not clear how pregnancy and high levels of s E2 affect 5-HIAA levels; previous studies in pregnant women and animal data show conflicting findings. Animal research in rodents and non-human primates report conflicting evidence. Exogenous estrogens may increase 5-HT and 5-HIAA in the central nervous system (Bethea et al., 2002). However, one study also found that the early postpartum period was associated with an increased 5-HIAA in the dorsal raphe nuclei, which projects serotonergic neurons to most of the brain (Holschbach and Lonstein, 2017). Meanwhile, one human study found that 5-HIAA CSF levels did not differ between pregnant women and non-pregnant controls (Altemus et al., 2004) and another small study found that 5-HIAA was higher in pregnant women compared to non-pregnant women, and that 5-HIAA levels increased in response to natural onset of labor (Spielman et al., 1985). Hormone changes during menstrual cycle does not seem to map

on to 5-HIAA(Eriksson et al., 1994). Relatively little is known about the role of 5-HIAA in perinatal mental health, but non-human primate studies show that low CSF 5-HIAA levels in animals with maternal experience increases healthy maternal behavior, while this relationship is reversed in first-time mothers (Pawluski et al., 2019). Thus, CSF measurements of 5-HIAA may provide insights to some of the potential serotonergic risk mechanisms for perinatal depression in pregnancy, including SERT expression.

The serotonin 4 receptor

The serotonin 4 receptor (5-HT4R) is a postsynaptic excitatory Gs-coupled receptor, which uses adenylate cyclase activation and increased cAMP formation for intracellular signaling (Sharp and Barnes, 2020). Intriguingly, animal data suggest that brain SERT is closely linked to the 5-HT4R function in the brain, including complimentary effects on 5-HIAA concentrations (Jennings et al., 2012; Cecilie L. Licht et al., 2010). In the human central nervous system, 5-HT4R density is particularly high in striatum and to a lesser extent cortical regions and subcortical regions such as hippocampus (Beliveau et al., 2017). Prefrontal 5-HT4R has been implicated in the regulation of neuronal firing in the dorsal raphe nuclei which in turn modulates overall serotonergic tonus in the brain (Murphy et al., 2021). Rodent data indicate that administration of selective serotonin reuptake inhibitors (SSRIs), a class of antidepressant drugs that block SERT, lead to a reduction of brain 5-HT4R levels. Further, 5-HT4R agonists may augment SSRI actions in the brain (Cecilie L. Licht et al., 2010) . Similarly, pharmacological human studies using *in vivo* molecular neuroimaging indicate that long-term blocking of SERT with SSRIs leads to a reduction in 5-HT4R brain binding (Haahr et al., 2014). Further, the low-expression variant of 5-HTTLPR is associated with lower 5-HT4R brain levels in healthy individuals (Fisher et al., 2012).

Rodent studies point towards anxiolytic, pro-cognitive and antidepressant properties of 5-HT4R, including anhedonia (Murphy et al., 2021). In individuals with a familial risk for depression, 5-HT4R binding is reduced in striatum, a key region in reward processing (Madsen et al., 2015). Thus, 5-HT4R may be involved in anhedonia. In the context of PND, this is particularly relevant as anhedonia is a key characteristic in depression with early postpartum onset (Putnam et al., 2017). Similarly, data from our lab point towards a reduced 5-HT4R brain binding in patients with major depression (Koehler-Forsberg et al., 2019). Also the anxiolytic properties of 5-HT4R agonists are

interesting given the high prevalence of perinatal anxiety symptoms and their association with postpartum risk for depression (Blackmore et al., 2016; Putnam et al., 2017; Robertson et al., 2004). Preclinical data shows that 5-HT4R may play a role in estradiol regulation of prolactin release in the rat pituitary and may thus play a role in sex-hormone functions (Papageorgiou and Denef, 2007). While there is currently limited knowledge about the role of 5-HT4R in hormone transitions we know that women exhibit lower 5-HT4R brain binding in limbic areas compared to men, suggesting that 5-HT4R availability in the brain may be regulated by sex-hormones (Madsen et al., 2011). In healthy men, 5-HT4R brain binding is inversely correlated with testosterone (Perfalk et al., 2017). Further, the use of oral contraceptives has been associated with lower 5-HT4R levels in healthy women, suggesting that the downregulation of endogenous sex-steroids may affect the 5-HT4R levels (Larsen et al., 2020). Cognitive symptoms, i.e., disturbances in thinking and emotion processing, also appear to be a key feature of PND. One study found that working memory capacity (i.e., the ability to briefly hold and manipulate information in the mind) improves in relation to the perinatal transition in healthy antepartum women, but worsens in the women who developed a perinatal depression (Hampson et al., 2015). Data from animal models indicate that 5-HT4R may be involved in regulation of hippocampal plasticity, which may mediate some of the proposed pro-cognitive effects (Hagena and Manahan-Vaughan, 2017). Notably, human molecular imaging studies in healthy populations support a link between 5-HT4R levels and memory function (Haahr et al., 2013; Stenbæk et al., 2017) and recent intervention studies using prucalopride, a partial 5-HT4R agonist, show that direct stimulation of the 5-HT4R system improves performance on memory tasks in humans (de Cates et al., 2021; Murphy et al., 2019). A partial 5-HT4R agonist is also currently under consideration as a potential drug for treatment of Alzheimer's disease (Roux et al., 2021). Thus, 5-HT4R is interesting in the context of perinatal mood regulation, especially as it is intimately linked to SERT availability as well as cognitive functioning. Further, it may have antidepressant properties particularly relevant for the perinatal transition, that seems to be mediated through hippocampal plasticity.

Hippocampal plasticity and sex-hormone transitions

Hippocampus is a subcortical brain region located deep in the temporal lobe that retains neuroplastic properties in adult life (Denoth-Lippuner and Jessberger, 2021; Roux et al., 2021). It

plays a key role in memory function, but has also been implicated in affective disorders (Willner et al., 2013). Sex-steroid receptors are highly expressed in the hippocampus and especially estrogens appear to affect synaptic remodeling and neurogenesis in hippocampus (Been et al., 2021; Galea et al., 2006; Gould et al., 2000; Pawluski et al., 2010, 2009; Pawluski and Galea, 2006; Sheppard et al., 2019; Yankova et al., 2001). Both human and animal data indicate that hippocampal volume changes and sex-hormone fluctuations may be intimately linked, as dynamic estradiol changes over the menstrual cycle are associated with rapid synchronous changes in hippocampal volume (Barth et al., 2016; Lisofsky et al., 2015; Pletzer et al., 2018; Protopopescu et al., 2008; Woolley and McEwen, 1992). Overall, these studies find that hippocampal volume peaks just before ovulation when estradiol concentrations are highest. Similarly, estrogens, in form of hormone replacement therapy, appear to increase hippocampal volume and improve cognitive functioning in perimenopause in a dose-dependent manner (Albert et al., 2017; Lord et al., 2008; Sohrabji, 2005). However, for hormone changes in the perinatal period, the pattern is different. From preconception to the postpartum period the human brain undergoes functional and structural reorganization in areas important for infant care, including a decrease in hippocampal volume (Hoekzema et al., 2017; Kim et al., 2010; Lisofsky et al., 2019; Martínez-García et al., 2021; Oatridge et al., 2002). Unlike other pregnancy-related grey matter volume changes, hippocampal volume is normalized within two years (Hoekzema et al., 2017; Martínez-García et al., 2021). Although it is unclear from human studies if the volume reduction occurs during pregnancy or in the postpartum period, rodent studies indicate that the perinatal period is characterized by high hippocampal plasticity and suggests that a decrease in hippocampal volume may start in pregnancy and serve to fine-tune the brain for motherhood (Pawluski et al., 2021). The plastic changes in the perinatal period may have consequences beyond the current pregnancy and postpartum. Maternal experience seems to affect hippocampal plasticity long-term, with effects on hippocampal plasticity in subsequent pregnancies and later in life, where it appears to affect cognitive performance and predict the hippocampal plasticity in response to estrogen stimulation (Barha et al., 2015; Barha and Galea, 2011; Eid et al., 2019; Galea et al., 2014; Macbeth et al., 2008; Medina and Workman, 2020; Pawluski and Galea, 2006). Further, perinatal hippocampal plasticity may be involved in depressive behavior in animals (Baka et al., 2017; Galea et al., 2014; Pawluski et al., 2015; Zhang et al., 2016). One human study found that DNA methylation markers

of postpartum depression not only correlate with estradiol-induced changes in DNA methylation, but also that these methylation patterns related to hippocampal plasticity (Guintivano et al., 2014). Thus, hippocampal plasticity may be important in perinatal mental health. Intriguingly, human neuroimaging studies show that hippocampal function and communication with other brain areas also seems to be affected, as hippocampal resting-state functional connectivity is attenuated in PND (Deligiannidis et al., 2013). A similar pattern has been observed in our sex-hormone manipulation model, indicating that sex-hormone effects on hippocampal function and perhaps structure, may be relevant in risk for PND (Fisher et al., 2017). Data from depressed patients outside the perinatal period, indicate that depression is associated with a reduced hippocampal volume (McKinnon et al., 2009; Schmaal et al., 2016; Videbech and Ravnkilde, 2004). Hence, the emergence of depressive symptoms in response to sex-steroid transitions may be associated with hippocampal volume changes as well.

As described previously, serotonergic signaling plays a role in hippocampal plasticity (Alenina and Klempin, 2015). There are some indications that estrogen affects hippocampal plasticity in interaction with serotonergic signaling; for instance evidence points to estrogens exerting neuroprotective properties in interaction with serotonergic signaling during perimenopause (Amin et al., 2006; Bethea et al., 2002; Frokjaer et al., 2010). Similarly, perinatal hippocampal plasticity seems to be affected by SSRIs specifically (Pawluski et al., 2019).

Thus, changes in hippocampal plasticity during hormone transitions may play a key role in the emergence of depressive symptoms, possibly dependent on serotonergic signaling.

Neuroimaging methods

Positron emission tomography

Positron emission tomography (PET) is an imaging technique that allows for in vivo quantification and mapping of molecular targets, for instance neuroreceptors in the brain (Heurling et al., 2017; Lammertsma, 2019). PET radio ligands are molecules that bind a target of interest and are labelled with a radioactive isotope that decays by positron emission, e.g. ^{11}C. Importantly, the PET tracer cannot exert any pharmaceutical effect on the target of interest, which is accomplished through extremely low concentrations (tracer dose). The radioactive isotope decays at a fixed rate (half-life) and the emitted positron is quickly annihilated by an electron in the surrounding tissue. The annihilation reaction emits two gamma rays of 511keV at an angle of 180 degrees, which can be

recorded by crystal detectors in a PET scanner. From the recorded radioactive decay, reconstruction algorithms can estimate the location of the decay. After correction for random events, scatter and absorption in bone and tissue, a PET image is available.

Kinetic modelling

Dynamic PET-scans (scanning over time), allow for estimation of time-activity curves (TACs), i.e., the radioactivity (in MBq per volume tissue) over time for a brain volume of interest (VOI). Through compartmental modelling of the tracer kinetics, TACs can be used to quantify the ligand binding for a region of interest. Specific binding potentials (bound to the target of interest) in a target tissue can be quantified relative to free or total plasma concentration derived from arterial blood sampling (Heurling et al., 2017). However, for radioligands with a suitable reference region (a region devoid of specific binding to the target receptor) it is possible to use a reference-tissue model instead, such as the simplified reference tissue model (SRTM), and avoid arterial cannulation (Lammertsma and Hume, 1996). This allows for the estimation of the non-displaceable binding potential (BPND) i.e., specifically bound ligand relative to the non-displaceable uptake (free ligand and non-specific binding to various proteins), which is the product of receptor density and affinity for the ligand (Heurling et al., 2017). In addition to a reference region devoid of specific binding to the target receptor, the SRTM assumes that target and reference tissues have same volume of distribution of non-specifically bound ligand, the kinetics in target and reference tissue can be modelled by a one tissue compartment model, and the blood contributes negligible to the signal. SRTM modelling of the PET tracer ^{11}C-SB207145 with cerebellum as refence region has been validated for quantification of 5-HT4R availability (Marner et al., 2010, 2009).

Structural MRI

Structural magnetic resonance imaging (MRI) is a non-invasive non-radioactive imaging technique that produces high-resolution three-dimensional (3D) anatomical images (McRobbie et al., 2017). The technique is based on electromagnetic properties of atomic nuclei, mainly hydrogen protons in water molecules. When a tissue is placed in a strong magnetic field within the scanner (B0) the protons attempt to align with the magnetic field and precess around the direction of the field. The rotating net magnetization of these protons is aligned with the main field B0. When a

radiofrequency (RF) pulse is transmitted, energy is transferred to the protons and the rotating net magnetization of the protons is pushed away from the B0 field at a desired angle. The RF pulse creates a transversal magnetic field (B1) which is perpendicular to B0. After the RF pulse is transmitted (echo time), it is possible to measure oscillating currents induced by the rotating transversal B1 field with a receive coil. After the RF pulse is terminated, protons dephase resulting in an exponential decay of the magnetization in the transverse B1 field, the relaxation time for this phenomenon is T2. Due to tissue dependent field-inhomogeneities in the B0 field, what is really observed after a RF pulse is the effective transversal magnetization time constant: T2*. Further, energy transferred to the protons by the RF is transferred to the surroundings, allowing the protons to return to their equilibrium position and thus restoring the longitudinal magnetization, the relaxation time for this phenomenon is T1. Both T1 and T2 vary between tissues. The time between successive excitation pulses is the repetition time, TR. By applying gradients, i.e., spatially linear variation in the static field strength along B0, it is possible to affect the frequency of the MR signal, which can be used to distinguish between MR signals at different positions in space. The signal can then be translated in to 3D images. By variating the basic parameters and taking the T1's and T2's for different tissues in to account, it is possible to optimize MR sequences to image all kinds of tissues. Finally, the signal to noise ratio can be improved increasing the strength of the magnetic field, measured in Tesla. Most modern MR scanners for neuroimaging use a 3 Tesla field, but higher field strengths are possible, although currently these are mainly used for specialized research purposes.

In this thesis we focus on data from the widely used T1-weighted anatomical images, which has a good spatial resolution and a good contrast between different brain tissues (grey matter, white matter, CSF). The resulting images can be parcellated in to discrete neuroanatomical structures by automatic segmentation procedures, which allows for an estimation of the size (volume) of the structure. There are multiple options for automatic segmentation procedures, and their details are beyond the scope of this thesis. Version 7.1 of the commonly used software Freesurfer (http://surfer.nmr.mgh.harvard.edu/), uses a mesh-based atlas and a Bayesian modeling framework to obtain volumetric segmentations without the need for skull-stripping, which provides reliable subcortical segmentations, including of the hippocampus (Fischl, 2012; Fischl et al., 2002; Puonti et al., 2016; Sederevičius et al., 2021).

Motivation, aims and hypotheses

Study 1

Women have an increased risk for depressive episode and subclinical levels of mental distress in relation to sex-hormone transitions. Some studies indicate that hippocampal volume and plasticity may play an important role in this, in a manner that may depend on estradiol and serotonergic signaling. In a pharmacological risk model for depression, we have previously shown that sex-hormone manipulations with a GnRHa can trigger subclinical depressive symptoms in healthy women. Further, the emergence of these symptoms was associated with the magnitude of the GnRHa-induced decline in estradiol, which seemed to be modified by an increase in SERT brain binding. However, data on hippocampal volume changes in this risk model had not yet been evaluated. Thus, in Study 1, we evaluated if GnRHa intervention was associated with hippocampal volume changes and if these changes mapped on to the GnRHa-induced estradiol decrease and the emergence of depressive symptoms.

We hypothesized that:

- Hippocampal volume would decrease in response to GnRHa relative to placebo.
- The GnRHa-induced net estradiol decrease would be associated with reduced hippocampal volume in interaction with change in brain SERT binding, such that increasing SERT and a greater estradiol decrease was associated with a greater hippocampal volume decrease
- The GnRHa-induced decrease in hippocampal volume would be positively associated with the emergence of depressive symptoms.

Study 2

The hormone changes from late pregnancy to early postpartum provides a natural model for studying potential contributions of estrogens and related neurobiological changes to risk mechanisms for perinatal depression and mental distress. Some studies indicate that there is a window of vulnerability within the first 4-8 weeks postpartum, which may depend on neurobiological changes. Previous studies have not found any association between depressive symptoms and absolute levels of estrogens. However, serotonergic signaling, especially SERT putatively plays a role in the emergence of depressive symptoms. *In vivo* imaging of brain SERT is

not possible in pregnant women for ethical reasons, but CSF levels of 5-HIAA can be a used as an indirect marker of SERT induction/availability. Thus, in Study 2, we evaluated how estradiol changes from late in pregnancy to five weeks postpartum and antepartum CSF levels of 5-HIAA were associated with the emergence of mental distress symptoms postpartum and whether SERT expression capacity played a role in this.

We hypothesized that:

- High 5-HIAA antepartum and a large ante- to postpartum decrease in estradiol would be associated with more subclinical mental distress symptoms in postpartum women
- High 5-HIAA in interaction with a large decrease in estradiol would increase postpartum mental distress
- The peripartum change in estradiol would increase mental distress in interaction with the high-expressing 5-HTTLPR variant relative to the low expressing 5-HTTLPR variant

Study 3

The perinatal period is characterized by high hippocampal plasticity, which may be associated with mental distress. However, the role of sex-hormone fluctuations and serotonergic signaling in perinatal hippocampal plasticity remains largely unknown. The 5-HT4R system has been proposed as a promising antidepressant target that may in particular be relevant for hippocampal-related cognitive impairments. However, little is known about its association with sex-hormones and its role in PND. In Study 3, we evaluated how 5-HT4R binding and hippocampal volume mapped on to mental distress postpartum, and if hippocampal volume was associated with estradiol changes and 5-HT4R binding.

We hypothesized that:

- Healthy postpartum women would have a higher 5-HT4R brain binding than healthy female controls
- Healthy postpartum women would have a smaller hippocampal volume than healthy female controls
- Postpartum mental distress would be associated with brain 5-HT4R availability in interaction with change in estradiol, such that a large decrease in estradiol would be associated with more mental distress symptoms.

- 5-HT4R brain binding postpartum was positively associated with antepartum 5-HIAA levels
- Hippocampal volume postpartum would be inversely related to postpartum mental distress
- Hippocampal volume would be associated with brain 5-HT4R availability in interaction with change in estradiol, such that a large decrease in estradiol would be associated with a smaller hippocampal volume.

Methods

Study 1

Participants and design

The study population was recruited for a previously published study (Frokjaer et al., 2015).
63 healthy premenopausal women were included in a double-blind, placebo-controlled intervention study, approved by the local ethics committee (protocol-ID: H-2-2010-108). All participants gave written informed consent.
Inclusion criteria: regular menstrual cycle with a length of 23-35 days, no use of hormonal contraceptives, no history of neurological or psychiatric disorders, including premenstrual dysphoric disorder, no alcohol or substance abuse and a normal physical, neurological and gynecological examination.
Participants were block randomized to receive either placebo (saline injection) or a gonadotrophin releasing-hormone agonist (GnRHa). Block randomization was done with respect to the serotonin transporter-linked polymorphic region (5-HTTLPR) genotype, to minimize any bias from 5-HTTLPR (Frokjaer et al., 2015). Before intervention, participants completed baseline assessment which included structural MR scans, PET scan, blood samples and a HAMD-17 depression interview. Baseline assessments were on average completed on menstrual cycle day 5.2±2.1 (midfollicular phase). See **Figure 3**. for an overview of the study design. 60 women (placebo n=29, GnRHa n=31) completed follow-up, including MR scans, but three women did not: (1) One was excluded due to anovulation prior to intervention, (2) one woman conceived a child, and (3) one participant was unable to attend the MR scan at follow-up.

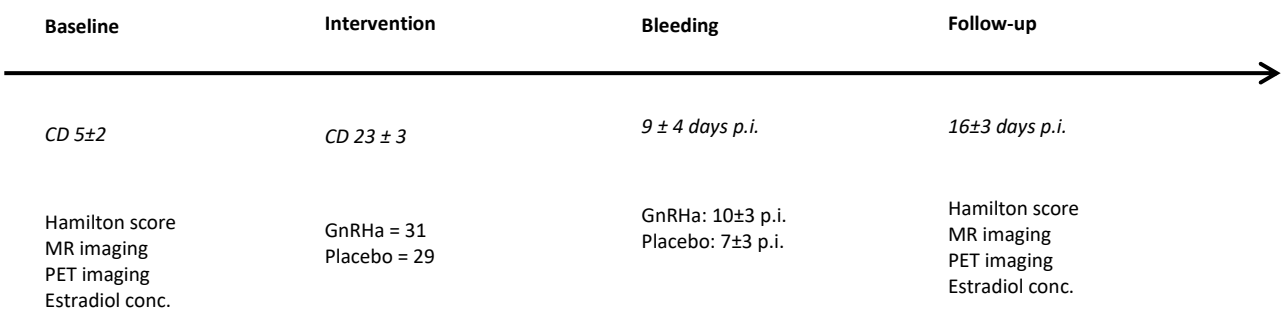


Figure 3. Overview of the study design, including neuroimaging. Note that there are small deviations in cycle days and intervention days from Figure 2, as the participants who completed MR-scans were slightly different from those previously published.

Intervention

A gynecologist, who was not otherwise involved in project, administered either placebo (saline) or a ZOLADEX, a biodegradable copolymer impregnated with 3.6 mg of goserelin (AstraZeneca, London, United Kingdom) to the participants on menstrual cycle day 22.75 ± 2.77. The post ovulation state was verified by the gynecologist with ultrasound.

Hormone analyses

Serum samples for estradiol analyses were collected at baseline and follow-up. Follow-up blood samples were on average collected 16.3±2.7 days after intervention. The serum samples were analyzed for estradiol concentrations within 24 hours of collection with an electrochemiluminescence immunoassay on Modular Analytics Serum Work Area equipment (Roche, Mannheim, Germany). The detection range was detection range 0.04 and 78.9 nmol/L. For statistical analyses, estradiol concentrations below the detection level were imputed to 0.04 nmol/L.

Structural MRI acquisition and hippocampal volume estimation

At baseline and follow-up, we acquired whole-brain T1-weighted structural images with a sagittal magnetization prepared rapid gradient echo (MPRAGE) sequence on a 3 Tesla Verio scanner with a 32-channel head array coil (Siemens, Erlangen, Germany). We used the following imaging parameters: TE = 2.32; TR = 1900; inversion time (TI) = 900 ms; flip angle= 9°; in-plane matrix: 256x256; in-plane resolution: 0.9x0.9mm; slice thickness = 0.9 mm; 224 slices, no gap.
An automatic subcortical segmentation of the resulting T1-weighted images was performed in the cross-sectional standard pipeline in Freesurfer version 7.1 (http://surfer.nmr.mgh.harvard.edu/; Fischl, 2012; Fischl et al., 2002; Puonti et al., 2016). Images were inspected for segmentation errors. If relevant, errors were manually edited before re-running the pipeline. Hippocampal volumes from the left and right hemisphere were extracted for statistical analysis.

[^{11}C]DASB-PET

[^{11}C]DASB binding potentials from neocortex, included in previous work by Frokjaer et al. (2015), were available through the CIMBI database (Knudsen et al., 2016). In brief, a bolus injection of [^{11}C]DASB was followed by a 90-minute dynamic PET scan on a high-resolution research tomography PET scanner (CTI/Siemens, Knoxville, TN, USA). The resulting images were movement corrected in AIR (Woods et al., 1992) and co-registered to the baseline T1-weighted MR image in SPM8 (http://www.fil.ion. ucl.ac.uk/spm/) for the purpose of automatic delineation of regions of interest (Svarer et al., 2005). Kinetic modeling was done with a modified reference tissue model (multilinear reference tissue model/multilinear reference tissue model 2) implemented in PMOD (version 2.9; PMOD Technologies, Zurich, Switzerland), with cerebellum (excluding the vermis) as reference region.

17-item Hamilton Depression Rating Scale interviews

17-item Hamilton Depression Rating Scale is a semi-structured interview for rating of depressive symptoms (HAMILTON, 1967) The resulting score range from 0-52, with higher scores reflecting more symptoms. HAMD-17 is not a diagnostic tool and there is not complete consensus on cut-offs. Some have suggested a score of eight as the lower cut-off for mild depression, while a moderate depression requires a score of at least 17 and a severe depression at least 24

(Zimmerman et al., 2013). In the current study, participants were interviewed by a trained clinician before and after intervention. Follow-up interviews were on average conducted 16.3±2.7 days after intervention.

Statistical analysis

Here, statistical analyses for main findings are detailed. For a full description of all analyses, please see Manuscript 1.

Prior to analyses, we evaluated covariates for non-normality and found that estradiol concentrations were not normally distributed. Hence, normally distributed log-transformed (base = 2) estradiol concentrations were used for statistical analyses. Change in estradiol was modelled as follow-up minus baseline.

To determine if there were any baseline effects irrespective of GnRHa sex-hormone manipulation, and thus specify our model, we evaluated if baseline hippocampal volume was associated with three potential variables: follicular phase estradiol concentrations, SERT binding potentials, and age. Inclusion of age as a covariate was motivated by the known age-related hippocampal atrophy (Fraser et al., 2015; Nobis et al., 2019; Sederevičius et al., 2021). All three variables were associated with hippocampal volume and thus considered relevant covariates in the main analyses. For further details please see Manuscript 1.

In line with the general analysis structure for the study (Frokjaer et al., 2015) *changes* from baseline to rescan in hippocampal volume, in estradiol concentration (within GnRHa group only) and in HAMD-17 were used in all main analyses. First, we evaluated if there was an effect of GnRHa compared to placebo in hippocampal volume change with a Welch two-sample t-test (Welch, 1947). Second, *within the GnRHa group only*, we used multiple linear regression models to determine associations between changes in hippocampal volume, change in estradiol, and the emergence of depressive symptoms. In the first model, we determined if GnRHa-induced changes in estradiol concentrations were associated with change in hippocampal volume, in interaction with or controlled for either SERT binding at baseline or change in SERT binding. Then, we evaluated if there was a main effect of change in hippocampal volume on the emergence of depressive symptoms *within* the GnRHa group, in interaction with or adjusted for the known effect of change in estradiol. All of these models were reviewed for potential contributions from age.

It was not possible to perfectly time MR scans with other study elements. Time delays of more than five days between MR scans and either blood samples, HAMD-17 or PET scans were considered potentially problematic for association analyses, since larger changes in hormone milieu might have occurred. Thus, we ignored data from individuals where these measures were more than five days apart in both groups at baseline (n = 3, all in GnRHa group) and at follow-up in the placebo group (n = 1). This cut-off was chosen as a conservative approach to the closeness of observations. They were not ignored for the GnRHa group at follow-up, as participants were considered fully downregulated in their estradiol concentrations for the entire period (Thomas et al., 1986). A total of four participants were ignored in change analyses (GnRHa: n = 3, placebo: n = 1). In between group analyses, all available data points were included.

Potential lateralization of the association between estradiol and hippocampal volume and between hippocampal volume and depressive symptoms was reviewed with a linear mixed effects model. A similar approach was used for group comparisons. We found no indication of a lateralization. Further, log-likelihood ratio tests supported that the inclusion of left and right hippocampus separately did not improve model fit compared to mean hippocampal volume pooled from both brain hemispheres. Thus, we used a mean hippocampal volume in all analyses.

The reported p-values were not adjusted for multiple comparisons as the study was driven by few *a priori* hypotheses and all other models were used for robustness analyses. p-values < 0.05 were considered statistically significant.

Study 2

Participants and design

An overview of the study design can be found in **Figure 4**.

For the purpose of CSF collection in relation to caesarean section (C-section), only women who were planned to deliver by C-section with spinal anesthesia were eligible for the study. Thus, recruited 100 healthy women, pregnant in week 38-42 and planned to deliver by C-section from obstetric departments at Rigshospitalet and Herlev Hospital, both Copenhagen University Hospital, Denmark. The study was approved by the local ethics comity in the Capital Region of Denmark (protocol H-18029563) and all participants gave written informed consent.

Inclusion criteria:

- 18-40 years old
- Planned C-section due to breech position of the child, previous C-section, previous myomectomy, obstructing fibroid tumor, previous rupture of the anal sphincter, or uncomplicated placenta previa.

Exclusion criteria:

- Previous or current severe somatic or psychiatric illness
- Pregestational BMI below 18 or above 35
- Severe postpartum bleeding
- Infant with severe illness
- Use of medications that affect the central nervous system
- Substance abuse
- Non-fluent in Danish
- Severe learning disabilities
- Impaired vision or hearing.

Screening included questionnaires on mental well-being, medical history and blood tests. Out of the included participants, eight had a psychiatric history of mild anxiety or depressive symptoms, including three cases of postpartum depression. Further two suffered either intrauterine fetal death or neonatal dead in relation a previous pregnancy. Two women had a possible thromboembolic episode in pregnancy and injected heparin, two women had a history of migraine, two had congenital urogenital anomalies, and one had a remitted thyroid disease. About one third of the participants were enrolled during the Severe Acute Respiratory Syndrome Coronavirus-2 (COVID-19) pandemic of 2020.

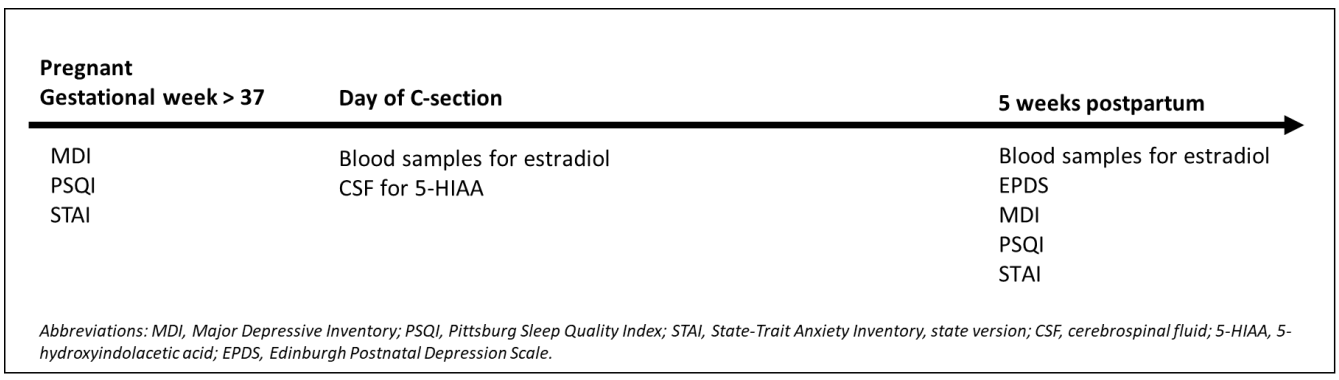


Figure 4. Overview of the study design.

Missing data

Out of the 100 women, four gave birth vaginally and were immediately excluded. Another 14 did not complete follow-up: three gave birth to infants with severe illness, two left the study for personal reasons, and we lost contact with nine before their week five appointment. Of the remaining 82, 19 had missing data at follow-up. One was enrolled under an older protocol version that did not include blood samples at week five. Five were not able to come in for blood samples due to COVID-19 related restrictions. Thirteen had non-compliance on questionnaires. Further, technical issues meant that quantification of CSF markers failed for two participants and genotyping for one participant. Only one woman developed significant depressive symptoms, but we were not able to describe a trajectory for clinically depressed cases and thus excluded her data from postpartum-, but not antepartum, analyses. Thus, complete data across all time points were available for 59.

Questionnaires and interviews

Levels of mental distress and depressive symptoms were quantified with questionnaires across the perinatal period. The main outcome was Edinburgh Postnatal Depression Scale (EPDS) score at week five. Antepartum, questionnaires were used for screening purposes, including the Major Depressive Inventory (MDI), State Trait Anxiety Inventory (STAI) and Pittsburgh Sleep Quality Index (PSQI). These questionnaires were repeated at week five postpartum. We used the Mini-International Neuropsychiatric Interview (M.I.N.I.) to make sure that no participant had an undiagnosed severe psychiatric disorder.

Edinburgh Postnatal Depression Scale (EPDS)

Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987) is a 10-item questionnaire that targets self-reported depression symptoms in the postpartum period, each item is rated from 0-3, resulting in a score range of 0-30, with higher scores reflecting more symptoms. We used a Danish validated version of the Edinburgh Postnatal Depression Scale (EPDS; Smith-Nielsen et al., 2018), which suggests that a postpartum score of 11 or more can be used for screening purposes. The

Danish version has not yet been validated for antenatal use; thus we did not administer it in pregnancy.

Major depressive inventory (MDI)

Major Depressive Inventory (MDI, Bech et al., 2001), is a scale for self-reported depressive symptoms and can be used for both diagnosis in accordance with ICD-10 (World Health Organisation, 1992) and DSM-IV (Del Barrio, 2016) criteria and for quantification of symptoms severity. It consists of 10-items and has a possible score range from 0-50, higher scores indicate more depressive symptoms. Cut-off for mild depression is a score of at least 21, for moderate to severe it is 26 (Bech et al., 2015).

State Trait Anxiety Inventory (STAI)

State Trait Anxiety Inventory (STAI; Spielberger et al., 1983) is a 40-item questionnaire for quantification of self-reported anxiety symptoms, each item is rated on a 4 point Likert scale. STAI is divided in a 20-item scale for trait anxiety symptoms and a 20-item scale for state anxiety symptoms. For each subscale, possible scores range from 20-80, with higher scores corresponding to more symptoms. We only used the state subscale in the current study, which rates symptoms at the exact moment it is completed. It can be used for diagnostic purposes, for pregnancy and postpartum cut-offs of 40 and 34, respectively, have been suggested (Tendais et al., 2014).

Pittsburg Sleep Quality Index (PSQI)

Pittsburg Sleep Quality Index (PSQI; Buysse et al., 1989) consists of 19 self-rated questions grouped into seven component scores, each weighted equally on a 0-3 scale. The component scores are summed to a global PSQI score, with a range of 0-21. Higher scores indicate worse sleep quality and scores above 5 indicate disturbed sleep. In the current study a version that concerned the past two weeks sleep was used.

Mini-International Neuropsychiatric Interview (M.I.N.I.)

For the purpose of screening for undiagnosed severe previous psychiatric disorders, we used the Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998), which is a

structured diagnostic psychiatric interview for both DSM-IV and ICD-10. The interview was completed at week five postpartum.

Estrogen analyses

Blood samples for estradiol (E2) analyses were collected before C-section (median: 0 days, range: [0; 2]) and repeated five weeks postpartum (median: 35 days, range: [15; 66]). Serum samples were centrifuged 30 minutes after collection and transferred to -20 °C within 3 hours from collection. Concentrations of estradiol were measured simultaneously with the estrogens estrone and estriol in serum samples as described in Frederiksen et al., 2020. Briefly, estrogens including E2 were purified from 200 µL thawed serum sample by liquid–liquid extraction using heptane/ethyl acetate. This was followed by analysis on a Dionex UltiMate 3000 UHPLC system with integrated Transcend TLX TurboFlow sample preparation system, coupled with triple quadrupole mass spectrometer (TSQ Quantiva) from Thermo Scientific controlled by Aria MX 2.2 and Xcalibur 4.0 software. The TurboFlow liquid chromatography tandem mass spectrometry system was equipped with a loading Cyclone-P TurboFlow column, for further sample extraction, followed by an analytical Kinetex® Phenyl-Hexyl column, for chromatographic separation of estrogens. The tandem mass spectrometry system was equipped with a heated electrospray ionization source running in negative mode. The Total duration time 5.50 minutes

Cerebrospinal fluid samples and neurotransmitter analyses

Cerebrospinal fluid (CSF) was collected as a part of the anesthetic procedures for the C-section. Briefly, anesthesiologists collected 0.5-1 ml of CSF during spinal anesthesia, which was immediately transferred to dry ice and subsequently stored at -80 °C.

CSF concentrations of 5-hydroxyindoleacetic (5-HIAA) , serotonin (5-HT), norepineprine (NE), 3-methoxy-4-hydroxyphenylglycol (MOPEG), and homovanillic acid (HVA) were assayed by high-performance liquid chromatography with electrochemical detection, as detailed in (Weikop et al., 2007). Briefly, 10 µL of the samples was injected onto a Prodigy C18 column (100 x 2 mm I.D., 3-µm particle size, YMC Europe, Schermbeck, Germany) at flow rate of 0.15 mL/min. The mobile phase consisted of 55 mM sodium acetate, 1 mM octanesulfonic acid, 0.1 mM Na2EDTA and 7% Acetonitrile, adjusted to pH 3.7 with 0.1 M acetic acid. It was degassed using an on-line degasser.

The electrochemical detection was conducted with an amperometric detector (Antec Decade from Antec, Leiden, The Netherlands) with a glassy carbon electrode set at 0.7 V, with an Ag/AgCl as reference electrode. The output was recorded on a computer program system CSW (Data Apex, Prague, The Czech Republic). This was used to calculate the peak areas.

5-HTTLPR genotyping

Analysis was performed on full blood samples drawn prior to C-section and stored at −20 °C. We used a modified version of a previously published method (Kalbitzer et al., 2010). The full description can be found in Manuscript 2. Briefly, DNA was extracted from blood with a Chemagic 360-D (PerkinElmer, Waltham, Massachusetts) according to the manufacturer's guidelines. We performed 5-HTTLPR (SLC6A4; rs774676466) genotyping by PCR amplification with the forward primer 5'-TAATGTCCCTACTGCAGCCC-3' and reverse primer 5'-GGGACTGAGCTGGACAACC-3' and separation of the fragments by gel electrophoresis. For statistical analyses, participants were dichotomized into long allele homozygotes (LL) or carriers of the short allele (S-carrier).

Statistical analysis

Here, statistical analyses for main findings are detailed. For a full description of all analyses, please see Manuscript 2.

As EPDS only is validated for postpartum use in Danish, we were not able to adjust for antepartum levels of mental distress. We kept this structure for STAI, PSQI and MDI as well, since antepartum scores might be biased from stress prior to C-section and thus have introduced noise. However, we used linear mixed-effects models with an unstructured covariance matrix to describe mental distress changes across the whole perinatal period.

Estradiol was log-transformed (log2) to ensure normally distributed data. The ante- to postpartum change in estradiol was modelled as postpartum minus antepartum (ΔE2). Thus, a large ante- to postpartum decrease corresponded to a large *negative* value.

We used multiple linear regression models to test if either 5-HIAA or ΔE2, controlled for age, parity and 5-HTTLPR genotype, was associated with EPDS at week five. 5-HIAA and ΔE2 were not mutually adjusted, to ensure that each model included all available date. Potential age, parity and 5-HTTLPR genotype interaction effects with either 5-HIAA or ΔE2, were evaluated in similar

models. However, we used a model with both 5-HIAA and ΔE2 to evaluate if they interacted in promoting mental distress and if not, to ensure that the effects were independent. Age, parity and 5-HTTLPR genotype were chosen because they are known risk factors for perinatal depression (Aasheim et al., 2012; García-Blanco et al., 2017; Gavin et al., 2005; Munk-Olsen et al., 2014; Muraca and Joseph, 2014; Sanjuán et al., 2008; Tavares Pinheiro et al., 2013). Number of days from C-section to follow up was also considered as a potential covariate. Log-likelihood-ratio test indicated that only parity and 5-HTTLPR were relevant covariates, but we included age as well since primiparous women were younger than multiparous women. In addition to the main analyses, we evaluated if 5-HIAA was associated with mental distress antepartum, to rule out that postpartum associations were carried over from late in pregnancy. Supplementary analyses, to roughly evaluate if a 5-HIAA antepartum 5-HIAA might be a result of estradiol effects on MAO activity in pregnancy, included antepartum associations between E2 and monoaminergic neurotransmitters or their metabolites, including 5-HIAA, 5-HT, 3-methoxy-4-hydroxyphenylethyl-glycol (MOPEG), homovanillic acid (HVA) and norepinephrine (NE). For 5-HIAA and 5-HT we also included 5-HTTLPR in the model. Similarly, we evaluated if 5-HT, MOPEG, HVA and NE equally well explained mental distress postpartum, as an indication of MAO activity being the underlying mechanism. P-values were not adjusted for multiple comparisons. $p < 0.05$ was considered significant.

Study 3

Participants and design

Participants included in Study 2 were invited to a neuroimaging program, approved under the same ethics protocol. An outline for this program can be found in **Figure 5**. It included ^{1}C-SB207145 PET scan for cerebral 5-HT4R quantification and structural MRI for hippocampal volume quantification. Out of the 100 women included in Study 2, 31 consented to participate in the neuroimaging program. Out of these 31 women, all completed a structural MRI scan and 23 also completed a ^{11}C-SB207145 PET scan, between week 3-5 postpartum. Study 3 reports from this imaging subgroup.

Inclusion criteria and exclusion criteria were as in the main study, with the addition of two exclusion criteria: contraindications for MRI scans and exposure to radioactivity of > 10 mSv within

the last year. Participants included in the imaging program continued to follow the study program for Study 2. To provide a reference for the data, healthy female controls from other projects conducted at approximately the same time as Study 3, were chosen from the CIMBI database (Knudsen et al., 2016) based on the following criteria: Female sex, age 18-40 years, no use of oral contraceptives, ^{11}C-SB207145 images and/or structural MRI scans acquired on the scanners used for the postpartum women. 24 women fitted the criteria and had undergone ^{11}C-SB207145, of these, 22 were scanned on the same MR scanner as the postpartum women. All were screened for mental and physical illness as a part of their participants in other projects.

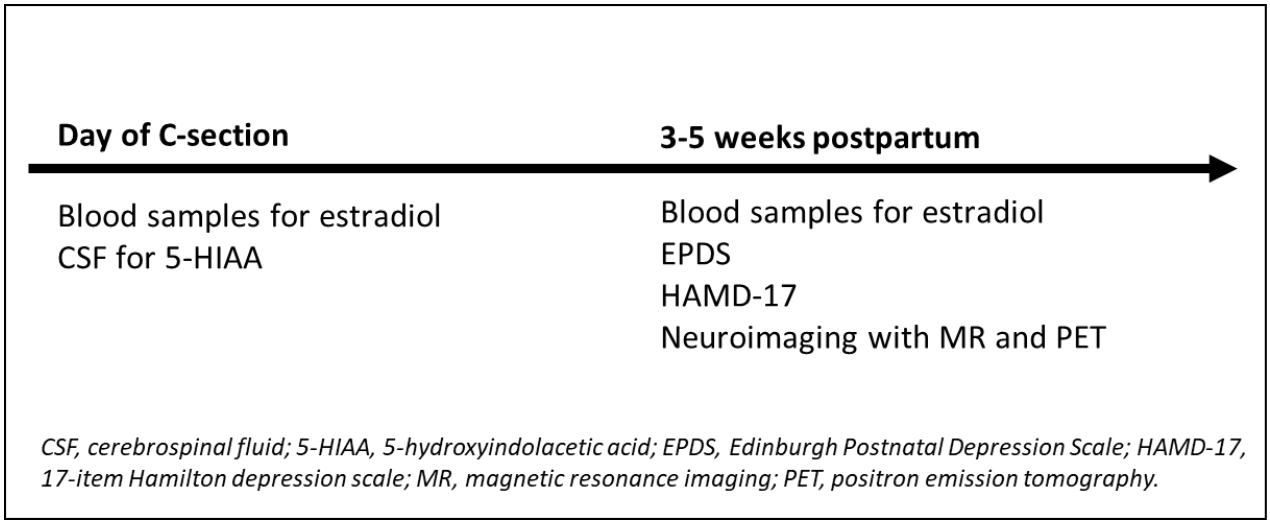


Figure 5. Neuroimaging program overview of design. 100 women were included in a cohort study across late pregnancy and early postpartum (see Figure 4). A subgroup of 31 women was invited to participate in a neuroimaging program that included MR and PET scan in the early postpartum period.

Mental distress quantification

We used the HAMD-17 interview (described under Study 1) to rate perinatal mental distress in the imaging subgroup. Participants were interviewed by a trained clinician or a student under supervision. The women, who underwent both PET and MR scans, were interviewed in relation to their PET scan, while the rest were interviewed on the day of the MR.

To ensure that any uniquely postpartum symptoms also were captured, we included data from the EPDS (described in Study 2), which the participants completed five weeks postpartum as a part of their participation in Study 2.

Blood samples and serotonergic markers in cerebrospinal fluid

Participants included in Study 3 continued to be a part of Study 2. Thus, samples for estimation of antepartum and postpartum serum estradiol concentrations and antepartum CSF 5-HIAA levels were collected and analyzed exactly as described under Study 2. Postpartum blood samples were collected on the PET scan day or, for those who only underwent MR, on the MR scan day. In line with Study 2, estradiol concentrations were log-transformed (base = 2) and change in log-transformed estradiol concentrations (ΔE2), was modelled as follow-up minus baseline, i.e., a large negative value reflects a large decrease.

Structural MRI acquisition and hippocampal volume estimation

T1 -weighted whole-brain structural MRI scans (MPRAGE sequence) were acquired on a 3 Tesla Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil with the following image acquisition parameters: T1 scans: inversion time = 900 ms, TE = 2.58 ms, TR = 1900ms, flip angle = 9°, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap. All MR images were evaluated by a specialist in neuroradiology in order to rule out any pathological conditions. Two participants had anatomical variants within the normal spectrum (arachnoid cyst and persistent cavum septum pellucidum). For the purpose of hippocampal volume extraction, T1 weighted images underwent automatic subcortical segmentation in the standard cross-sectional pipeline in FreeSurfer 7.1 (https://surfer.nmr.mgh.harvard.edu/, Fischl et al., 2002b; Puonti et al., 2016). Quality control steps followed the ENIGMA (http://enigma.usc.edu/) protocol. Additionally, all images were manually inspected for segmentation errors. We extracted the estimated volume of left and right hippocampus for statistical analysis. T1-weighted images were also segmented into white matter, grey matter and CSF in SPM8 (http://www.fil.ion. ucl.ac.uk/spm/) for delineation of PET regions of interest.

Acquisition of PET data in breastfeeding women

For 5-HT4R imaging we used the radioligand ^{11}C-SB207145. Radioactive labelling with ^{11}C is particularly suitable for PET imaging in breastfeeding women, as it has a short half-life of about 20 min. Prior to injection of the tracer, but after the radioactive calibration source had been removed, mothers were allowed to have the infant with them in the scanner room, if they wanted to. Mothers who decided to bring their child with them to the scanner room were encouraged to breastfeed about 30-45 minutes before initiation of the PET scan. No later than 15 minutes before initiation of the PET scan, mothers left the infant in the care of a close relative or a member of the research team. To ensure that the infants not were exposed to radioactivity, this person cared for the child at a different location until 10 half-lives had passed.

PET data acquisition and kinetic modelling

We administered a bolus injection of ^{11}C-SB207145 over 20 seconds and conducted a 120-minute dynamic PET scan on a high-resolution research tomography PET scanner (CTI/Siemens, Knoxville, TN, USA). To reduce radiation exposure, we aimed at an injected radioactive dose of 500 MBq (mean: 503.3 MBq, SD: 10.7).

Reconstructed and attenuation corrected PET images were motion corrected in AIR 5.2.5 (Woods et al., 1992) and co-registered to the corresponding MR image in SPM8 (http://www.fil.ion.ucl.ac.uk/spm), before automated regional delineation in Pvelab (Svarer et al., 2005). Regional TACs were extracted for kinetic modelling in PMOD (PMOD version 3.0, Zurich, Switzerland). BPNDs for neostriatum, neocortex (a volume-weighted average of all cortical regions) and hippocampus, quantified with the SRTM (Lammertsma and Hume, 1996), were extracted for statistical analysis.

Statistical analysis

Here, statistical analyses for main findings are detailed. For a full description of all analyses, please see Manuscript 1.

Group differences in age and MDI was evaluated with Wald type t-tests, while group differences in the distribution of 5-HTTLPR genotype was evaluated with Fisher's exact test. A t-test revealed that non-postpartum women were significantly younger than postpartum women. Previous

studies of the 5-HT4R with ^{11}C-SB207145 have reported minor age effects (Madsen et al., 2011) and there is a well-established decrease in hippocampal volume with age (Nobis et al., 2019). Thus, we used linear regression models controlled for age to evaluate if the postpartum women were significantly different from non-postpartum women in terms of 5-HT4R brain binding and hippocampal volume.

For analyses within the postpartum group, we used multiple linear regression models to evaluate our hypotheses. We first determined if hippocampal volume or 5-HT4R brain binding was associated with mental distress at week five postpartum, evaluated with HAMD-17 and EPDS. Data from Study 2, which these participants were recruited from, suggested that perinatal ΔE2, antepartum 5-HIAA, age, parity and 5-HTTLPR genotype might be potentially relevant covariates. Further, 5-HTTLPR has known effects on 5-HT4R brain binding (Fisher et al., 2012). Additionally, some women did not complete the MR scans for hippocampal volume on the same day as HAMD-17 and EPDS. The number of days between distress rating and MR scan was considered a potential covariate for the hippocampal volume analyses. We used the Akaike information criterion (AIC) for model selection to avoid overfitting of the model. Parity and ΔE2 were relevant covariates in models that associated 5-HT4R or hippocampal volume models with EPDS. Only parity was relevant in models associating 5-HT4R or hippocampal volume with HAMD-17, but we included ΔE2 to maintain the same analysis structure. Number of days between distress scoring and MR was a relevant covariate for associations between hippocampal volume and mental distress. Thus, we evaluated the association between mental distress and hippocampal volume in a model adjusted for parity, ΔE2 and days between scan and rating, and the association between mental distress and 5-HT4R brain binding in a model adjusted for parity and ΔE2.

Further, we used simple linear regression to evaluate if postpartum 5-HT4R brain binding was associated with antepartum 5-HIAA.

5-HT4R brain binding and ΔE2 in interaction effects on hippocampal volume postpartum, was evaluated in multiple linear regression models adjusted for age and parity, Age and parity status were considered relevant covariates, due to their potential effects on hippocampal volume (Hoekzema et al., 2017; Nobis et al., 2019), this was confirmed with AIC. MR scans were also of often conducted on a different day than PET and blood samples. Thus, we considered including the time difference between MR and PET/blood samples in this model, but AIC indicated that it did

not improve model fit. Follow-up analyses confirmed that it did not substantially change the results.

Reported p-values are not adjusted for multiple comparisons. $p < 0.05$ was considered significant.

Results

Here the main results for the thesis are presented. Please see Manuscript 1-3 for full details and results.

Study 1

Descriptive statics

Data on demographics, hormone levels and mean hippocampal volume for the two groups can be found in **Table 1.** The median age at inclusion was 22.4 years old (range: [18.4; 37.2]).

Table 1. Demographics, hippocampal volume and estradiol concentrations in the GnRHa and placebo group at baseline and follow-up. From Manuscript 1, Table 1.

	Baseline				Follow-up			
	GnRHa	Placebo	*p-value*	Effect size	GnRHa	Placebo	*p-value*	Effect size
Age *Mean±SD*	23.3±3.3	25.4±5	0.11[a]	0.43[c]	_	_	-	-
5-HTTLPR genotype LALA / S-carrier	10/21	10/19	1[b]	1.1 [0.33;3.70][d]	_	_	-	-
Mean hippocampal volume in mm3 *Mean±SD*	4282±364	4164±374	0.22[a]	0.32[c]	4256±319	4177±390	0.39[a]	0.22[c]
Estradiol concentration in nmol/L *Mean±SD*	0.19±0.09	0.19±0.11	0.96[a]	0.01[c]	0.07±0.03	0.36±0.23	< 0.001[a]	1.62[c]
Hamilton score *Mean±SD*	1.16±1.55	1.59±2.23	0.4[a]	0.22[c]	3.23±3.23	1.76±1.79	0.03[a]	0.56[c]

[a]: two-sample t-test
[b]: *Fisher's* exact test
[c]: Cohen's *d*
[d]: Odds ratio with 95% CI

Effect of intervention on hippocampal volume

Hippocampal volume decreased more in the GnRHa group, than in the placebo group, but this did not reach statistical significance (mean difference in change = 37.9 mm3, 95% CI: [-13.155; 88.956], p = 0.14, **Figure 6).**

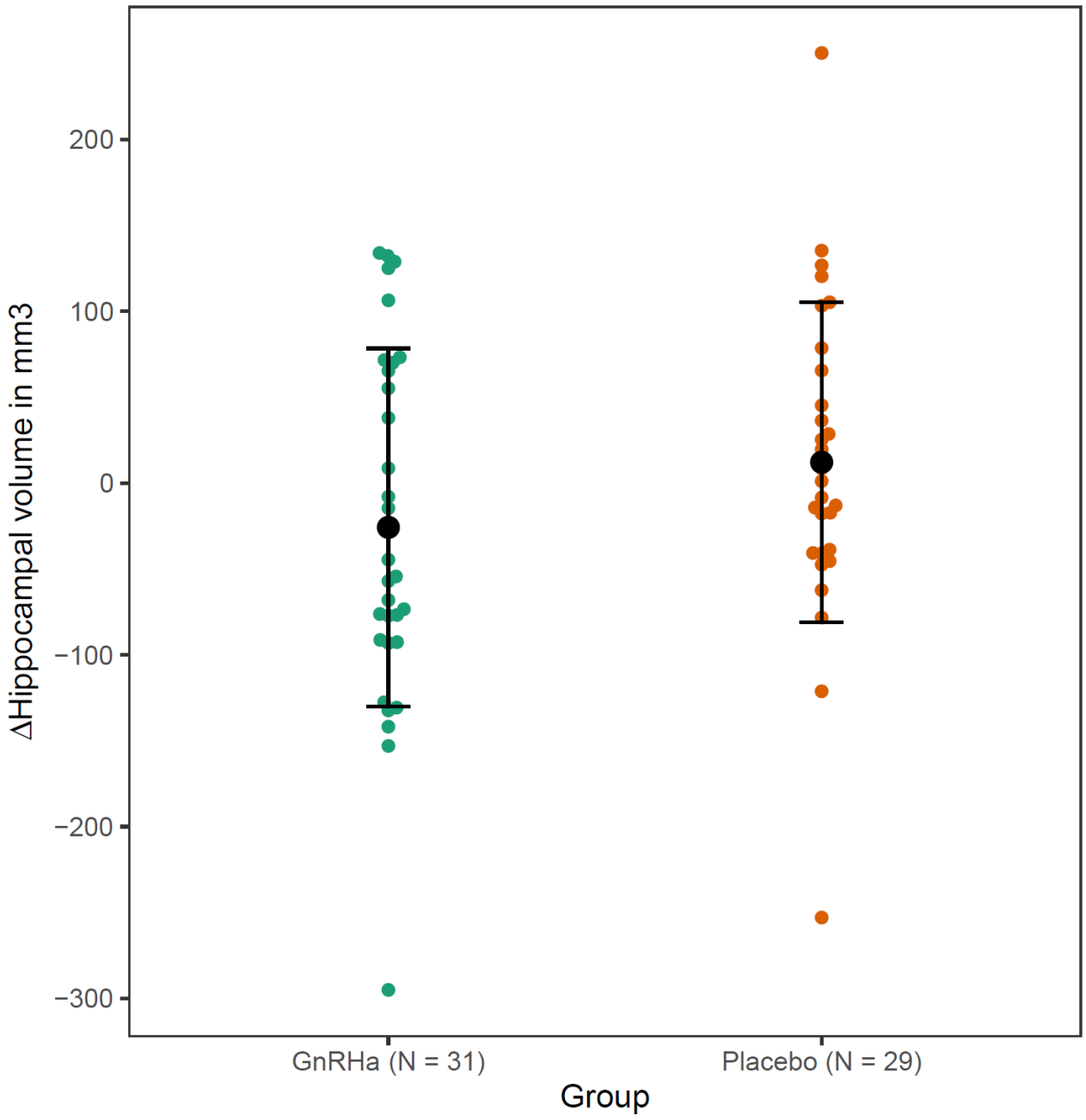


Figure 6. Differences in hippocampal volume change between the GnRHa and placebo group

In the GnRHa group, hippocampal volume decreased from baseline to follow-up (mean change = -27 mm^3), but the change was not significantly different from mean change in hippocampal volume

in the placebo group (mean change = 11 mm^3; (p = 0.14). Black dots represent mean change in hippocampal volume, error bars represent SD. Reproduced from manuscript 1.

Association between change in hippocampal volume and change in estradiol in the GnRHa group and interaction with serotonin brain binding

There were no interaction effects on hippocampal volume between change in estradiol and change in neocortical SERT binding ($\beta_{\Delta SERT}$ = 1420.30, 95% CI: [-1333.41; 4174.01], p = 0.3) or neocortical SERT binding at baseline (-234.6, 95% CI: [-1859.65; 1390.43], p = 0.77). However, change in estradiol was significantly associated with hippocampal volume, when controlled for SERT binding at baseline ($\beta_{\Delta estradiol}$ = 56.93, 95% CI: [1.98; 111.89], p = 0.04; $\beta_{SERT\_baseline}$ = 698.60, 95% CI: [22.97; 1374.24], p = 0.04, **Figure 7**). No such associations were found when change in SERT was included ($\beta_{estradiol}$ = 48.5, 95% CI: [-10.48; 107.48], p = 0.1; $\beta_{\Delta SERT}$ = 148.53, 95% CI: [-692.2; 989.27], p = 0.72). Adjusting for age made no difference in any of the models.

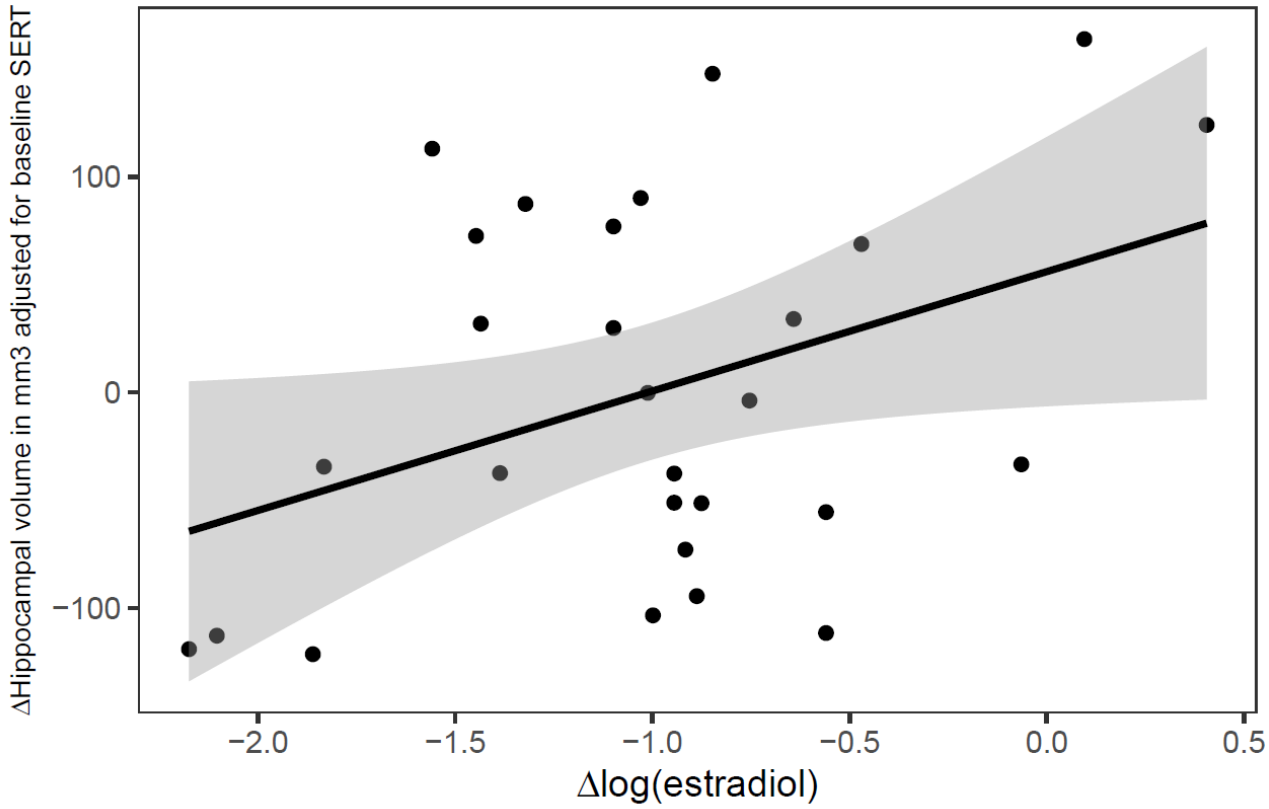


Figure 7. Association between estradiol and hippocampal volume in the GnRHa group. *GnRHa-induced change in estradiol concentration was positively associated with change in hippocampal volume ($\beta_{\Delta estradiol}$ = 56.93, p = 0.04), in a model adjusted for SERT binding at baseline (residuals are used to visualize hippocampal volume controlled for baseline SERT binding). Grey zone represents the 95% CI for the regression line. Reproduced from manuscript 1.*

Association between changes in hippocampal volume and the emergence of subclinical depressive symptoms in the GnRHa group

We previously published an increase in depressive symptoms, measured as Hamilton score, in response to GnRHa compared to placebo (Frokjaer et al., 2015). However, we observed no significant association between change in hippocampal volume and the emergence of depressive symptoms within the GnRHa group, adjusted for estradiol change ($\beta_{\Delta Hippocampus}$ = 0.01, 95% CI: [-0.006; 0.025], p = 0.21, **Figure 8**). Nor was change in estradiol (dichotomized with a median split) in interaction with hippocampal volume associated with depressive symptoms in the GnRHa group ($\beta_{estradiol\ large\ decrease\text{-}by\text{-}\Delta hippocampal\ volume}$ = 0.009, 95% CI: [-0.02; 0.04], p = 0.53). Inclusion of change in SERT or SERT at baseline did not substantially change these results (all p-values > 0.2).

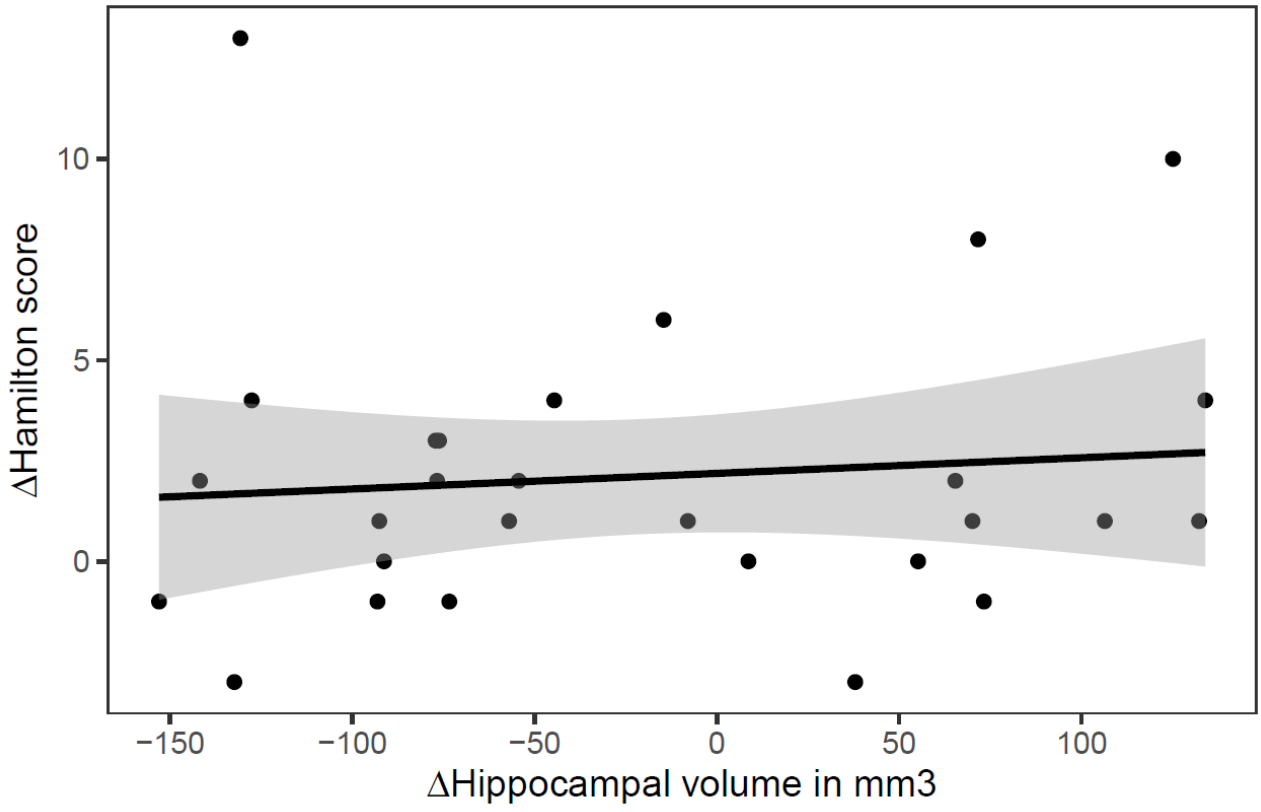


Figure 8. Association between change in hippocampus volume and the emergence of depressive symptoms. *We observed no significant association between change in hippocampal volume and the emergence of depressive symptoms, measured as change in Hamilton depression score ($\beta_{\Delta Hippocampus}$ = 0.01, p = 0.21). Black line represents regression line with 95% CI. Reproduced from manuscript 1.*

Study 2

Descriptive statistics

Sample characteristics are described in **Table 2**. At follow-up the number of participants with available data varied. Forty-four women underwent C-section due to fetal breech position; 39 due to previous C-section; 2 due to placenta previa; 7 due to previous rupture of anal sphincter; and 4 due to uterine fibroid or previous myomectomy. Scores of postpartum depressive symptoms were within the normal spectrum, although the average anxiety and sleep disturbances scores were close to or above suggested cut-offs.

MDI score decreased significantly across the antepartum to postpartum transition, (estimate: -2.09, 95% CI: [-3.33; -0.84], p = 0.001). A similar pattern was seen for STAI scores (estimate: -5.6, 95% CI:[-7.36; -3.85], p <0.001). PSQI score did not change significantly across the peripartum period (estimate: 0.17, 95% CI:[-0.55; 0.88], p = 0.65).

Table 2. Antepartum characteristics, neurotransmitter concentrations, peripartum mental distress and peripartum estradiol concentrations. From Manuscript 2, Table 1.

	Antepartum (n = 96)	**Week five postpartum (n = 82)**
Demographics		
Parity at inclusion (0 / ≥ 1 child)	38/ 57 [a]	-
Age	34 (3) / 34 (23; 41)	-

Mean (SD) / Median (range)		
5-HTTLPR genotype LL vs. S-carrier	28 / 67 [b]	-
Sex of child (Boy/Girl)	52/44	-
E2 and 5-HIAA		
Estradiol (E2) ***in pmol/L*** Mean (SD)	79387 (26630)	81 (152)[g]
Change in E2 ***(pmol/L)*** Mean (SD)	-	-78585 (27949)
5-HIAA ***in CSF in fmol/10µL*** Mean (SD)	1164.86 (366.71)[c]	-
Mental distress		
EPDS Mean (SD)	-	4.06 (4.22)[h]
MDI Mean (SD)	8.12 (5.25)	6.45 (6.07)[h]
STAI [e] Mean (SD)	32.51 (9.02)	27.17 (8.85) [h]
PSQI [f] Mean (SD)	6.74 (3.2)	7.07 (3) [h]
[a] n = 95, parity not logged for one dropout; [b] n = 95, analysis failed for one woman; [c] n = 94, analysis failed for two women; [e] n = 92; [f] n = 86; [g] n = 76; [h] n = 69		

Mental distress and associations with 5-HIAA, E2, 5-HTTLPR, age and parity

ΔE2 was significantly associated with EPDS at week five postpartum, unadjusted for 5-HIAA contributions ($\beta_{\Delta E2}$ = 0.73, 95% CI: [0.21; 1.25], p = 0.007; **Figure 9, A**). There was a parity-by- ΔE2 interaction effect, such that in multiparous women, a *large* decrease in E2 was associated with less

mental distress, measured with EPDS, while there was no association in the primiparous group ($\beta_{\Delta E2\text{-by-parity}}$ = 1.46, 95% CI:[-2.58; -0.33], p = 0.01). We found no significant interactions between ΔE2 and 5-HTTLR ($\beta_{\Delta E2\text{-by-5-HTTLPR}}$ = -0.81, 95% CI: [-2.12; 0.51], p = 0.22) or between ΔE2 and age ($\beta_{\Delta E2\text{-by-age}}$ = -0.02, 95% CI: [-0.17; 0.14], p = 0.84).

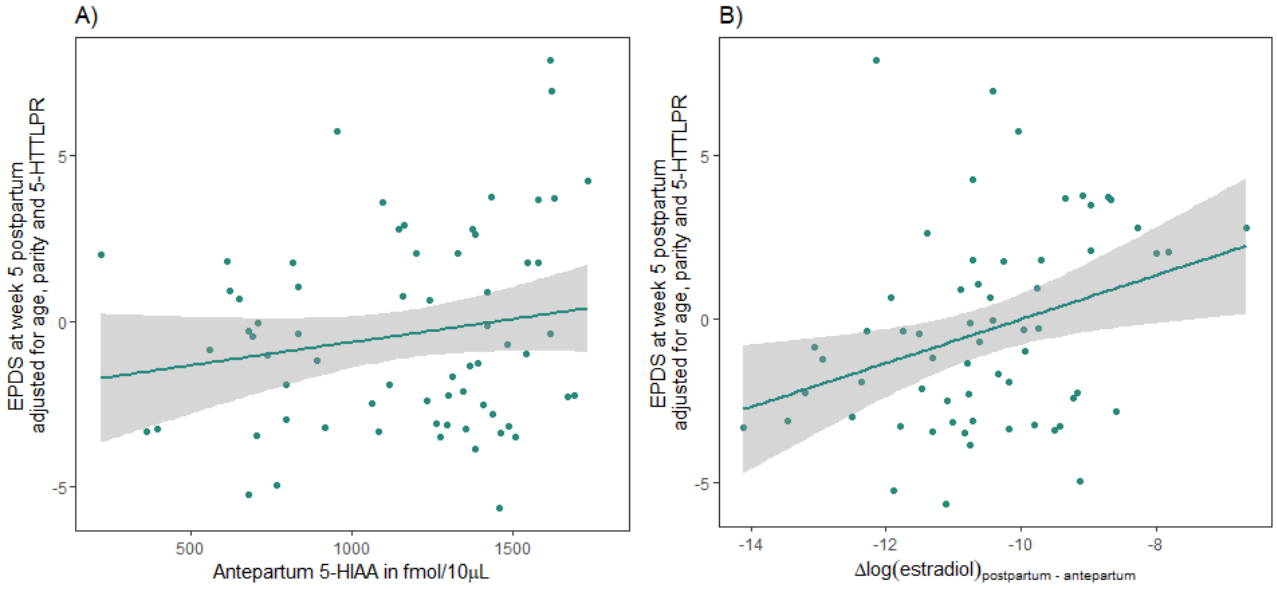
5-HIAA was positively associated with depressive symptoms with borderline significance, when not adjusted for ΔE2 contributions ($\beta_{5\text{-HIAA}}$ = 0.002, 95% CI: [-7×10-5; 0.004], p = 0.06, see **Figure 9, B**). In this model, 5-HIAA did not interact with age ($\beta_{5\text{-HIAA-by-age}}$ = 0.0003, 95% CI: [-0.0003; 0.0008], p = 0.36) or parity ($\beta_{5\text{-HIAA-by-parity}}$ = -0.002, 95% CI: [-0.006; 0.002], p = 0.32). Mutually adjusted (complete cases only, n = 59), 5-HIAA and ΔE2 associations with EPDS were similar, but less significant ($\beta_{\Delta E2}$ = 0.63, 95% CI: [0.09; 1.16], p = 0.02; $\beta_{5\text{-HIAA}}$ = 0.002, 95% CI: [-0.0004; 0.004], p = 0.12). 5-HIAA and ΔE2 did not interact with each other in promoting mental distress ($\beta_{5\text{-HIAA-by-}\Delta E2}$ = -0.0002, 95% CI: [-0.001; 0.001], p = 0.79). Adjusted for both 5-HIAA and ΔE2, first time mothers had significantly more symptoms on EPDS (β_{parity} = 1.66, 95% CI: [0.03; 3.29], p = 0.05), but neither age (β_{age} = 0.03, 95%cI: [-0.18; 0.25], p = 0.76) nor 5-HTTLPR were associated with EPDS ($\beta_{5\text{-HTTLPR}}$ = 1.44, 95% CI: [-0.41; 3.29], p = 0.12). Mental distress evaluated with MDI, STAI and PSQI is detailed in **Table 3**. Supplementary analyses indicated that 5-HIAA may have been associated with anxiety in pregnancy ($\beta_{5\text{-HIAA}}$: 0.006, 95% CI: [0.001; 0.01], p = 0.02), but not mood (0.001, 95% CI: [-0.002; 0.01], p = 0.36) or sleep (0.0004, 95% CI: [-0.002; 0.002], p = 0.69).

Table 3. 5-HIAA and ΔE2 associations with postpartum anxiety, sleep disturbances and mood, controlled for 5-HTTLPR, parity and age. From Table 2 Manuscript 2.

	MDI		***STAI***		***PSQI***	
	β (95% CI)	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*
5-HIAA	0.003 (0.0006; 0.006)	0.02	0.004 (8×10^{-5}; 0.008)	0.05	0.002 (-0.002; 0.002)	0.81
ΔE2	0.77 (0.07; 1.46)	0.03	0.21 (0.08; 2.34)	0.04	0.71 (0.27; 1.14)	0.002

Figure 9. A) Association between 5-HIAA concentrations in CSF and EPDS residuals after adjusting for age, parity and 5-HTTLPR ($\beta_{5\text{-HIAA}}$ = 0.002, p = 0.06). Linear regression line has 95% CI. **B)**

Association between Δlog(estradiol) and EPDS residuals after adjusting for age, parity and 5-HTTLPR ($\beta_{\Delta E2}$ = 0.73, p = 0.007). Linear regression line has 95% CI. From manuscript 2, Figure 2.



Supplementary analyses to illuminate potential contributions from MAO

Supplementary analyses for Study 2 of antepartum associations between estradiol and MAO degraded monoaminergic neurotransmitters or their metabolites are shown in **Table 4**. Data were available for 5-HIAA, serotonin (5-HT), norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MOPEG) and homovanilic acid (HVA). Supplementary analyses to evaluate the contributions from other MAO degraded neurotransmitters/metabolites to mental distress are detailed in **Table 5.**

Table 4. Associations between antepartum estradiol and CSF markers. Modified from supplementary Table 2, manuscript 2.

CSF marker	β_{E2}	*p*
5-HIAA	16.81 [a]	0.84
5-HT	1.62 [a]	0.74
NE	-16.55 [b]	0.11
MOPEG	66.69 [b]	0.16
HVA	-108.34 [b]	0.33

[a] *Adjusted for age and 5-HTTLPR contributions (neither had significant associations)* [b] *Adjusted for age (no significant associations)*

Table 5: 5-HT, MOPEG, HVA and NE associations with postpartum EPDS score, adjusted for ΔE2, age, parity and 5-HTTLPR. Modified from Supplementary table 3 and 4, manuscript 2.		
	β	*p*
5-HT	-0.01	0.47
MOPEG [a]	0.19	0.74
HVA [a]	0.77	0.07
NE [a]	0.81	0.13
[a] *Log-transformed due to non-normality*		

Study 3

Descriptive statistics

Healthy controls were significantly younger than postpartum women (mean difference: 9.45 years, 95% CI: [7.25; 11.65], p < 0.001), but did not differ in terms of 5-HTTLPR genotypes (postpartum women: 8 LL and 23 S-carriers; and healthy controls: 8 LL and 16 S-carriers; p = 0.57) or MDI (mean difference: 0.23, 95% CI: [-1.82; 2.29], p = 0.82). Sample characteristics for the postpartum women can be found in **Table 6.** Postpartum women were on average MR scanned 30 days (range: [20; 43]) postpartum and PET scanned 29 days postpartum (range: [15; 42]). On average, there were 5 days between MR scans and blood samples or HAMD-17 (range: [-13; 16]) and 6 days between MR and EPDS (range: [-21; 16]). On average, there were 7 days between MR and PET scans (range:[-13;16]).

Table 6. Characteristics for postpartum women. All 31 completed structural MR scans, 23 women also completed ^{11}C-SB207145 PET. From Manuscript 3, Table 1.				
	MR scanned (N = 31)		**PET scanned (N = 23)**	
	Antepartum	***Week five***	***Antepartum***	***Week five***
Age (years)	34 (4) [a]	-	34 (4) [a]	-

Parity (multiparous / primiparous)	-	16/15	-	11/12
Sex of child (male /female)		16/15		12/11
5-HTTLPR LL/S-carrier	8 / 23	-	5 / 18	-
MDI	7.16 (4.38) [a]	5.59 (3.24) [a]	7.70? (4.52) [a]	5.67 (3.23) [a]
HAMD-17	-	0.81 (1.8) [a]	-	1.04 (2.03) [a]
EPDS	-	3.93 (2.73) [a]	-	3.71 (2.8) [a]
E2 in pmol/L	83399 (30356) [a]	106 (231) [a]	85063 (30548) [a]	59 (57) [a]
ΔE2 in pmol/L	-	-83293 (30293) [a]	-	-85004 (30550) [a]
5-HIAA in fmol/10µL	1071.37 (420.47) [a]	-	1058.22 (449.49) [a]	-
[a] Mean (SD)				

Postpartum women compared to healthy controls: 5-HT4R binding, hippocampal volume and age

We found no significant differences between postpartum women and healthy controls in age-adjusted 5-HT4R brain binding in neostriatum (β_{group} = 0.13, 95% CI: [-0.35; 0.6], p = 0.6; β_{age} = -0.01, 95% CI: [-0.05; 0.03], p = 0.63, **Figure 10, A**), neocortex (β_{group} = 0.003, 95% CI: [-0.08; 0.09], p = 0.95; β_{age} = -0.003, 95% CI: [-0.01; 0.004], p = 0.42) or hippocampus (β_{group} = 0.1, 95% CI: [-0.09; 0.29], p = 0.31; β_{age} = 0.002, 95% CI: [-0.01; 0.02], p = 0.77). Group differences in 5-HT4R brain binding were similar without age-adjustment (neostriatum, mean difference: 0.22, 95% CI: [-0.52; 0.09], p = 0.17; neocortex, mean difference: 0.03, 95% CI: [-0.09; 0.03], p = 0.29; hippocampus, mean difference: 0.08, 95% CI: [-0.2; 0.05], p = 0.23).

We found no significant difference between postpartum women and healthy controls for mean hippocampal volume, adjusted for age (β_{group} = 154.86, 95% CI: [-122.07; 431.79], p = 0.27; β_{age} = 5.86, 95% CI: [-16.82; 28.54], p = 0.61; **Figure 10, B**) and not adjusted for age (mean difference: 100.42, 95% CI: [-77.96; 278.8], p = 0.26).

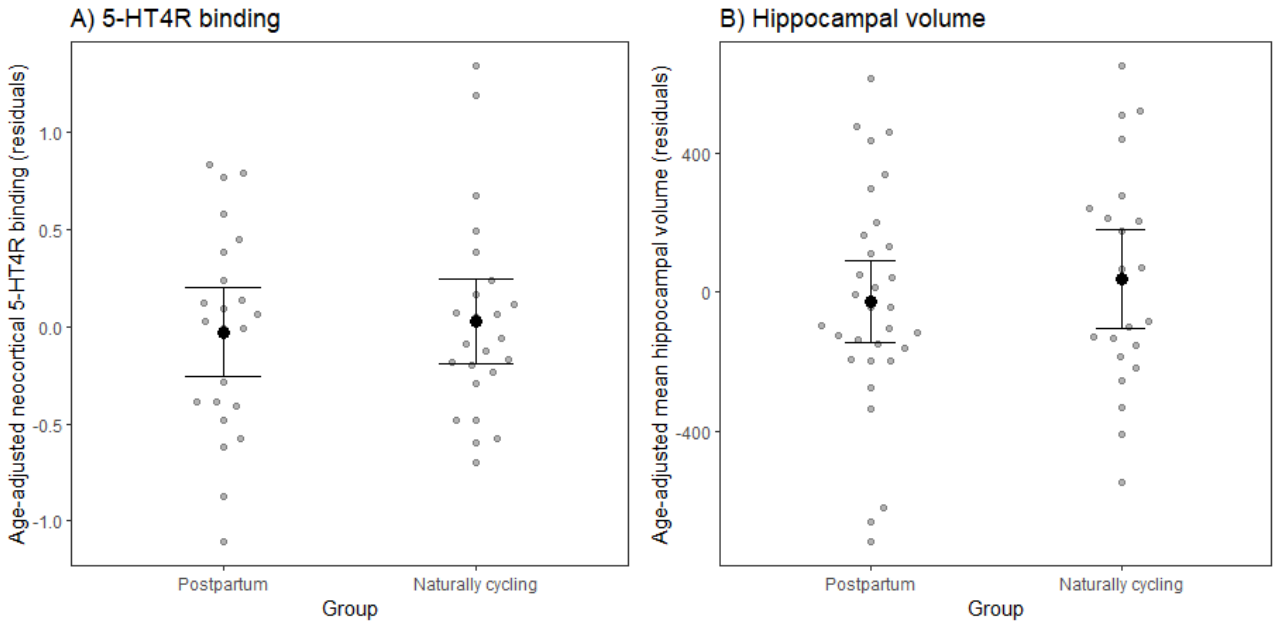


Figure 10. *Group differences in A) age adjusted 5-HT4R binding in neostriatum (residuals) and B) age adjusted hippocampal volume (residuals). We observed no significant difference between non-postpartum and postpartum women in terms of 5-HT4R brain binding (all p > 0.6) or hippocampal volume (p = 0.61).*

Mental distress, hippocampal volume, 5-HT4R binding and 5-HIAA in postpartum women

EPDS and HAMD-17 were not associated with 5-HT4R binding, adjusted for ΔE2 and parity, in neostriatum (HAMD-17: $\beta_{5\text{-HT4R}}$ = 0.52, 95% CI: [-1.32; 2.35], p = 0.56; EPDS: $\beta_{5\text{-HT4R}}$ = 0.94, 95% [-3.25; 1.36], p = 0.40) or neocortex (HAMD-17: $\beta_{5\text{-HT4R}}$ = 6.36, 95% CI: [-3.95; 16.67], p = 0.21; EPDS: $\beta_{5\text{-HT4R}}$ = -3.03, 95% CI: [-9.82; 15.89], p = 0.63). There was a positive association between EPDS or HAMD-17 and binding in hippocampus (HAMD-17: $\beta_{5\text{-HT4R}}$ = 4.65, p = 0.02; EPDS: $\beta_{5\text{-HT4R}}$ = 4.86, p = 0.03), but this was primarily driven by one single observation with relatively high score on HAMD-17 and EPDS. The association was not significant without this datapoint (HAMD-17: $\beta_{5\text{-HT4R}}$ = 1.87, 95% CI: [-2.49; 6.23], p = 0.38; EPDS: $\beta_{5\text{-HT4R}}$ = 2.95, 95% CI: [-2.85; 8.75], p = 0.27). 5-HT4R brain 5-HT4R brain binding and ΔE2 did not have any interaction effect on EPDS or HAMD-17, across all 5-HT4R regions (p > 0.12).

Hippocampal volume was associated with EPDS and HAMD-17 on a borderline significant level, controlled for the number of days between rating and MR scan, parity, and ΔE2 (HAMD-17: $\beta_{\text{hippocampal volume}}$ = 0.002, 95% CI: [0.0001; 0.004], p = 0.04; EPDS: $\beta_{\text{hippocampal volume}}$ =0.003, 95% CI: [-0.0001; 0.005], p = 0.06, **Figure 11 and 12**).

5-HT4R binding and antepartum 5-HIAA were not associated in neostriatum ($\beta_{5\text{-HIAA}}$ = -0.00006, 95% CI: [-0.0006; 0.0004], p = 0.81), neocortex ($\beta_{5\text{-HIAA}}$ = 0.00001,95% CI: [-0.0001; 0.0001], p = 0.75) or hippocampus (hippo: $\beta_{5\text{-HIAA}}$ = 0.0001, 95% CI: -0.0001; 0.0003], p = 0.28).

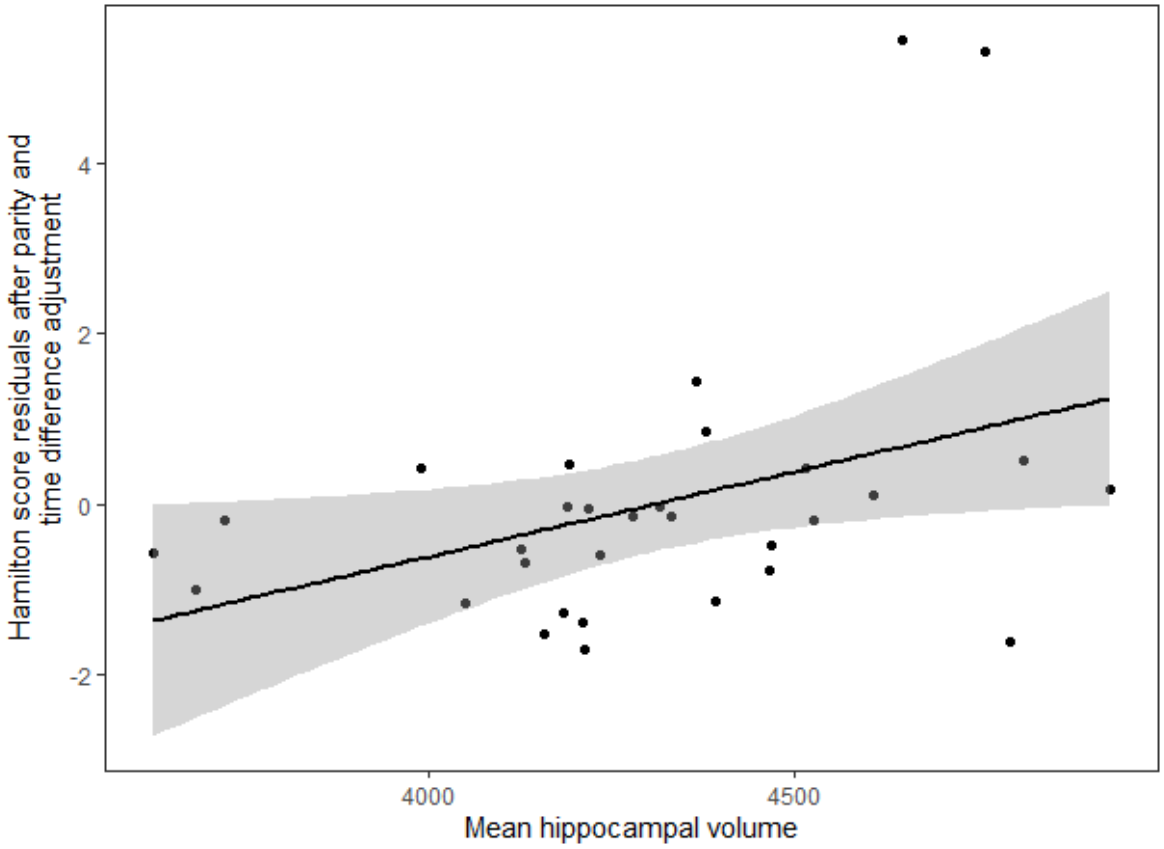


Figure 11. *There was a significant association between postpartum hippocampal volume and HAMD-17 ($\beta_{\text{hippocampal volume}}$ = 0.002, p = 0.04) adjusted for parity and ΔE2 (residuals).*

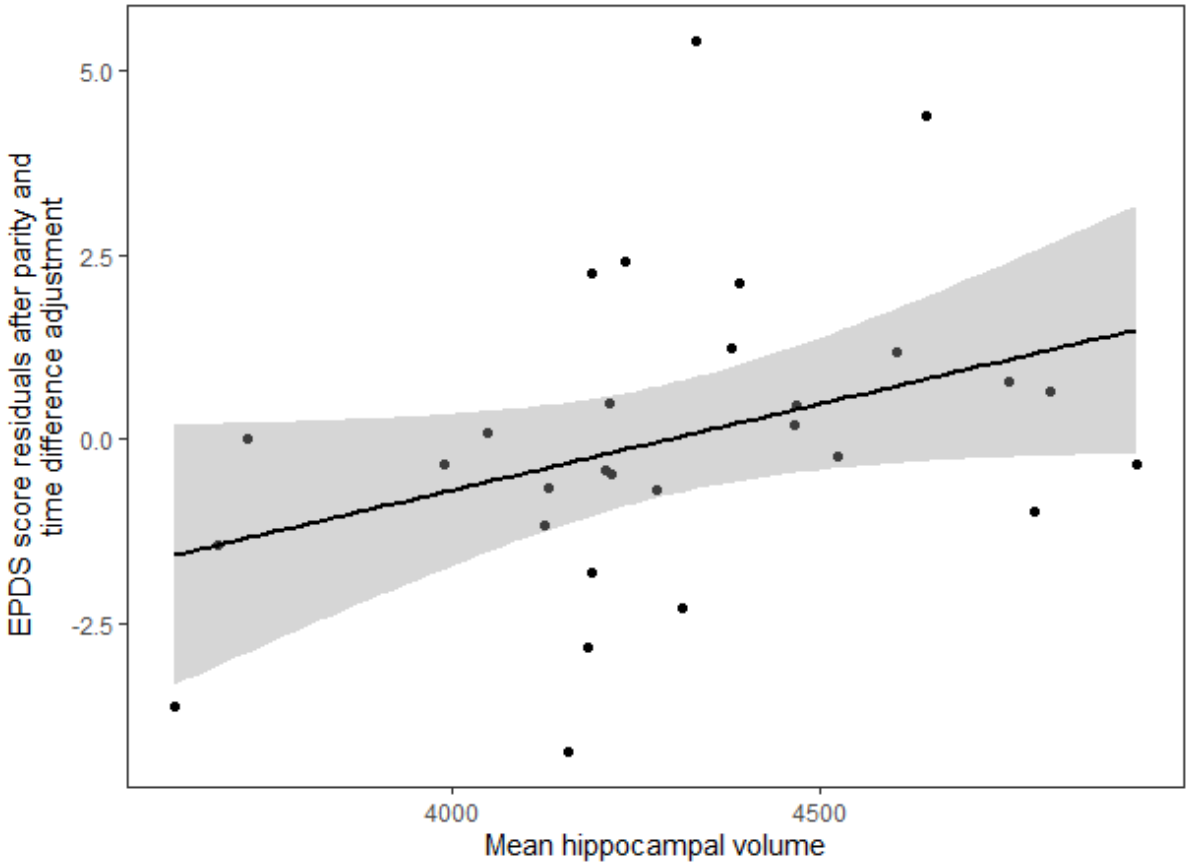


Figure 12. *There was a borderline significant association between postpartum hippocampal volume and EPDS ($\beta_{hippocampal\ volume}$ = 0.003, p = 0.06) adjusted for parity and ΔE2 (residuals).*

Association between hippocampal volume, 5-HT4R binding and estradiol in postpartum women

Within the postpartum group 5-HT4R binding in neostriatum ($\beta_{5\text{-}HT4R}$ = -11.74, 95% CI: [-322; 299], p = 0.94), neocortex ($\beta_{5\text{-}HT4R}$ = -343, 95% CI: [-2140; 1453], p = 0.69) and hippocampus ($\beta_{5\text{-}HT4R}$ =123, 95% CI: [-640; 886], p = 0.74) were not associated with hippocampal volume. Nor was estradiol, regardless of the 5-HT4R region in the model (all p > 0.41). However, 5-HT4R binding in neostriatum and hippocampus interacted with change in estradiol on hippocampal volume (neostriatum: $\beta_{5\text{-}HT4R\text{-}by\text{-}\Delta E2}$ = 258, 95% CI: [50; 466], p = 0.02; hippocampus: $\beta_{5\text{-}HT4R\text{-}by\text{-}\Delta E2}$ = 905.54, 95% CI: [358; 1453], p = 0.003, **Figure 13**). A similar, but less strong, effect was found for neocortex ($\beta_{5\text{-}HT4R\text{-}by\text{-}\Delta E2}$ = 985.97, 95% CI: [-85; 2057], p = 0.07). The direction of the effect was such that low

5-HT4R and a large decrease in estradiol or a high 5-HT4R and a small decrease in estradiol resulted in a higher hippocampal volume.

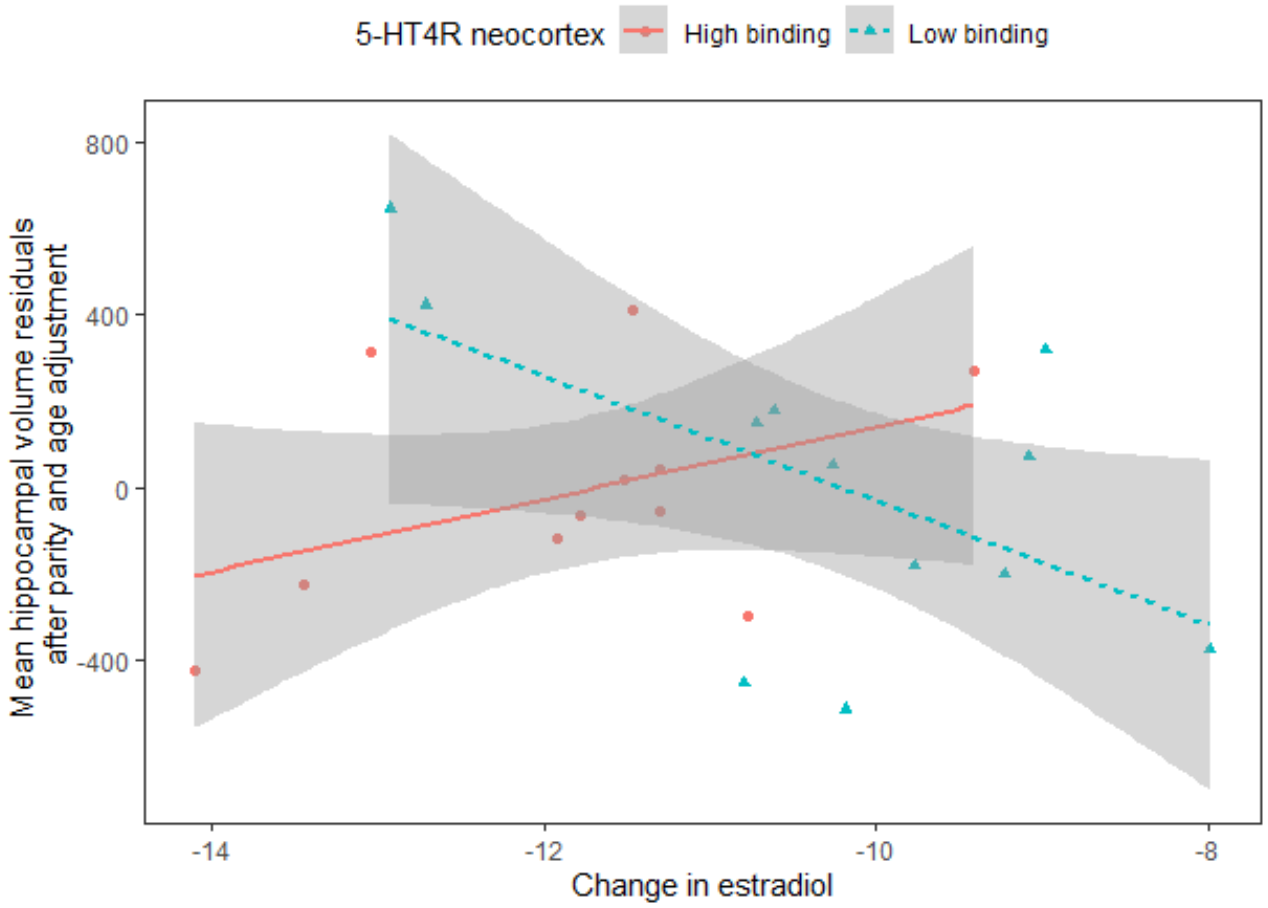


Figure 13. *Interaction effect between 5-HT4R binding in neostriatum and change in estradiol on hippocampal volume ($\beta_{5\text{-}HT4R\text{-}by\text{-}\Delta E2}$ = 258, 95% CI: [50; 466], p = 0.02). For the purpose of two-dimensional visualization, 5-HT4R binding in neostriatum was dichotomized with a median split, resulting in two arbitrary groups (high and low binding).*

Discussion

Study 1

GnRHa, estradiol and hippocampal volume

Contrary to our expectations, sex-hormone manipulation with GnRHa did not significantly reduce hippocampal volume, compared to placebo. Since the placebo group underwent MR at approximately the same timepoint in their menstrual cycle, the observed changes in this group does not represent fluctuations of hormonal levels. This suggests that hippocampal volume changes of the magnitude GnRHa can induce are of a size similar to non-estrogen related fluctuations in hippocampal volume. It should be noted that, while the difference between groups was non-significant, it was in the expected direction and magnitude as seen in depressed populations (Schmaal et al., 2016). However, the present sample may not allow sufficient power to detect effects of this size. In line with this, we confirmed the hypothesis that greater decrease in estradiol, adjusted for SERT availability, was associated with a greater decrease in hippocampal volume. This points towards a dose-response relation between estradiol fluctuations and variations in hippocampal volume. It harmonizes well with studies in naturally cycling women and animal models, where hippocampal volume is positively associated with endogenous estradiol concentrations and follows the fluctuations in estradiol across the menstrual cycle (Barth et al., 2016; Lisofsky et al., 2015; Woolley and McEwen, 1992). Similarly, it aligns with the neuroprotective effects of perimenopausal hormone replacement therapy on hippocampal volume (Albert et al., 2017; Lord et al., 2008; Sohrabji, 2005). Hormone transitions across pregnancy and the postpartum period are also associated with a net decrease in hippocampal volume (Hoekzema et al., 2017; Pawluski et al., 2016, 2009). However, it is not clear from human data if the hippocampal volume decrease is caused by the high levels in pregnancy or the reduction postpartum (Hoekzema et al., 2017), as grey matter volumes in general may be reduced in pregnancy (Oatridge et al., 2002) and animal data show that hippocampal volume may decrease already in pregnancy (Pawluski et al., 2016, 2009).

SERT contributions to GnRHa hippocampal volume changes

Although we previously found that SERT changes in interaction with estradiol changes were associated with the emergence of subclinical depressive symptoms, this did not map on to hippocampal volume changes in the GnRHa group. However, adjusted for estradiol contributions, low baseline SERT binding was associated with a greater GnRHa-induced decrease in hippocampal volume, suggesting that low SERT increased hippocampal sensitivity to changes in neurotrophic stimuli. This points towards a key role for serotonergic signaling in hippocampal plasticity, as observed by others (Alenina and Klempin, 2015; Kraus et al., 2017). It is possible that this relates to the association between 5-HT4R and SERT under stable conditions (Haahr et al., 2014; Cecilie L. Licht et al., 2010), but other factors are likely to involved, too. Increased hippocampal sensitivity to trophic stimuli may translate to genomic changes in the same manner as vulnerability to depressive symptoms during sex-hormone transitions (Guintivano et al., 2014; Mehta et al., 2019). However, the current data suggest that SERT may affect hippocampal volume and depressive symptoms through different mechanisms.

GnRHa-induced hippocampal volume changes and depressive symptoms

We were not able to confirm our hypothesis that the estradiol induced fluctuations in hippocampal volume mapped on to depressive symptoms. Although estradiol changes in the GnRHa group were associated with hippocampal volume, nothing indicated that hippocampal volume changes might mediate the previously published association between estradiol and depressive symptoms (Frokjaer et al., 2015). Such mediation effects were found for hippocampal resting state functional connectivity in a previous study, but it seems like the functional and structural changes in hippocampal volume not were linked (Fisher et al., 2017). Of course, we cannot rule out that the functional changes originated from a specific hippocampal subfield, which we unfortunately not were able to delineate in our noisy MR images of an older date. The results contrasts with data from clinical populations, where depressive symptoms are associated with a decreased hippocampal volume (McKinnon et al., 2009; Schmaal et al., 2016; Videbech and Ravnkilde, 2004). However, there may be large differences between experiencing short-term subclinical depressive symptoms and the full range of neurobiological changes associated with manifest depressive episode. Further, we may not have sufficient power to detect associations

between a very limited range of subclinical symptoms and hippocampal volume changes that are within the normal range.

Study 2

Generally, the participants in the study had a good mental health across the perinatal period, also better than expected from population studies (Meltzer-Brody et al., 2013). We observed a pattern where anxiety and depressive symptoms decreased from pregnancy to the postpartum, while sleep disturbances were unchanged. This may have been due to stress related to the C-section, however this pattern has also been observed in other studies (Evans et al., 2012; Heron et al., 2004; Klier et al., 2007; Matthey and Ross-Hamid, 2012).

Estradiol changes and mental health postpartum

As hypothesized, perinatal changes in estradiol were associated with postpartum mental distress, but in the opposite direction of what we expected, i.e., a large ante-to postpartum change resulted in less postpartum mental distress. This contrasts our findings in the sex-hormone manipulation model, where the magnitude of the decrease mapped on to the *emergence* of subclinical depressive symptoms (Frokjaer et al., 2015). However, as discussed below, this may be due to differences in the length and magnitude of the estradiol exposure, which may have divergent effects on neurobiological mechanisms, as is the case for SERT expression (Deecher et al., 2008; Lu et al., 2003). It seems like the mechanisms that maintain mental health respond positively to the abrupt decrease in estradiol in healthy postpartum women, perhaps signaling a return to "normal" neurobiological conditions. Disruption of such mechanisms may lead to depression in the postpartum period. This supports that estrogen sensitivity may play a key role, in line with genomic studies pointing towards estrogen sensitivity as a risk marker for PND (Guintivano et al., 2014; Mehta et al., 2014; Osborne et al., 2016). Differences in estrogen sensitivity may also explain why our data conflict with studies who report a negative association between depression and estradiol levels (Fan et al., 2009; O'Hara et al., 1991). This differential response to sex-hormones may be related to the reduced working memory reported in antepartum depression, which contrasts the increased working memory in healthy pregnant women (Hampson et al., 2015). Notably, our results partially agree with two studies of the early

postpartum period. Klier et al. (2007) reported that health pregnant women who developed postpartum depression, had higher early postpartum estrogen levels, and Heidrich et al. (1994) found that increased estradiol levels might map on to blues symptoms. Our results differ from the number of studies that have found no association between postpartum mood symptoms and estradiol (Chatzicharalampous et al., 2011; Mehta et al., 2014; Okun et al., 2011; T. Abou-Saleh et al., 1998). However, methodological differences such as sample size, timing of the postpartum blood sampling, degree of clinically relevant symptoms, and absolute estradiol levels versus changes may account for this. Further, we here use a highly sensitive quantification method, which may have revealed subtle associations driven by the most extreme estradiol concentrations (in both directions). Especially older studies may have missed such associations. Estradiol changes seemed to be particularly beneficial for women who were parous at inclusion. This suggests that previous pregnancies may have resulted in an altered hormone sensitivity, perhaps related to genomic PND risk markers and/or long-term changes in hippocampal plasticity (Barha and Galea, 2011; Hoekzema et al., 2017; Mehta et al., 2014).

Serotonergic contributions to perinatal mental health

Contrary to our expectation, 5-HTTLPR played no role in perinatal mood, alone or in interaction with estradiol. This contrasts the reported increased risk for postpartum depression in women with high-expressing variants of 5-HTTLPR (Sanjuán et al., 2008; Tavares Pinheiro et al., 2013). However, we cannot rule out that such an effect exist, but that we do not have sufficient power to detect it. Further, it may not map on to the subtle changes in distress in our participants, but only clinical levels of depression. There were no indications of an interaction effect on mental distress between estradiol changes and 5-HIAA. Thus, if we consider 5-HIAA a proxy for SERT expression in pregnancy (Carpenter et al., 2003; De Bellis et al., 1993; Potter et al., 1985; Y et al., 1997), our results do not support that SERT interacted with estradiol changes in promoting mental distress. This does not align with results from our sex-hormone manipulation model (Frokjaer et al., 2015). However, 5-HIAA late in pregnancy was directly associated with postpartum subclinical mental distress, although only on a borderline significant level. Assuming that high 5-HIAA reflects high cerebral SERT levels, increased brain SERT may have carried over from pregnancy to the postpartum period and increased the susceptibility to develop mental distress symptoms.

Supplementary analyses indicated that the neurobiological changes related to high 5-HIAA may have been established already late in pregnancy, where they resulted in anxiety symptoms, and further unfolded by the ante-to postpartum transition. This aligns well with the increased risk for depression found in women with antepartum anxiety (Robertson et al., 2004). If so, high CSF 5-HIAA in pregnancy may reflect that brain SERT not was appropriately downregulated in response to the high levels of endogenous estradiol in pregnancy (Lu et al., 2003; Sumner et al., 2007). This phenomenon may be similar to the rigid SERT regulation associated with depressive symptoms during seasonal changes (Mc Mahon et al., 2016). However, 5-HIAA is also regulated by MAO-A degradation of 5-HT, which responds to estrogens (Lokuge et al., 2011). *In vivo* imaging of MAO-A in women with postpartum depression or mental distress symptoms, that indicate that MAO-A levels postpartum may play a key role in perinatal mood (Sacher et al., 2015). In this study, we found no associations between estradiol late in pregnancy and MAO degraded monoamines or their metabolites (5-HT, 5-HIAA,NE, MOPEG, HVA), suggesting that estradiol in pregnancy had no major impact on neurotransmitter degradation through monoamine oxidases. Nor would a high 5-HIAA in pregnancy correspond well with human studies, that find a higher brain MAO-A distribution early postpartum, where sex-steroid levels are low (Sacher et al., 2010). Similarly, supplementary models found no significant effects of non-serotonergic monoamines or metabolites on EPDS, including MOPEG which the result of MAO-A degradation of norepinephrine. Thus, based on the available data, MAO-A does not seem to be responsible for the association between high 5-HIAA in pregnancy and mental distress. However, with the available data, we cannot determine what changes that may have occurred in these CSF markers postpartum.

Study 3

Postpartum serotonin 4 receptor binding

Postpartum women did not differ from healthy controls in terms of 5-HT4R brain binding. We were surprised to find this, since 5-HT4R is reduced during oral contraceptive use, a state associated with blunted endogenous estradiol dynamics, and thus seem to be sensitive to sex-hormones (Larsen et al., 2020). Further, numerous other serotonergic markers respond to estrogens and sex-hormone transitions in both animal and human studies (Bethea et al., 2002; Deecher et al., 2008; Lokuge et al., 2011; Lonstein, 2019; Moses-Kolko et al., 2008; Pawluski et al.,

2019; Sacher et al., 2015, 2010). Both human and animal data indicate an intimate link between 5-HT4R and SERT (Fisher et al., 2012; Haahr et al., 2014; Jennings et al., 2012; Cecilie Löe Licht et al., 2010). However, the perinatal transition is characterized by dramatic changes that may induce multiple counterbalanced changes in the serotonergic system and result in 5-HT4R levels similar to those seen in healthy controls. Thus, we do not know if 5-HT4R remained stable across pregnancy. However, if 5-HT4R did not remain stable across pregnancy, it may have adjusted rapidly to the postpartum hormone and neurobiological milieu to ensure a healthy return to a non-pregnant brain state. In support of this, we did not observe an association between postpartum 5-HT4R brain binding and antepartum CSF 5-HIAA, although, as noted above, both may be related to SERT levels. Thus, acute neurobiological changes across the perinatal period may have disrupted the link between SERT and 5-HT4R. 5-HT4R brain binding did not map on to postpartum subclinical depressive symptoms, except in the hippocampus. This association was, however, driven by a single individual with high mental distress scores and did not translate to other regions, thus we suspect that it may be a spurious effect. If not, it suggests an association between increased 5-HT4R and distress. This is in the opposite direction of what is observed in non-postpartum depression (Koehler-Forsberg et al., 2019).

Postpartum hippocampal volume

We expected that the reported reduction in hippocampal volume across pregnancy and the postpartum in healthy women would result in a lower hippocampal volume in postpartum women, compared to women who had not recently given birth (Hoekzema et al., 2017), but found no significant difference. However, other studies evaluating pregnancy related changes in grey matter volumes have not reported any changes in hippocampus, perhaps because such changes are small and hard to detect (Lisofsky et al., 2019; Oatridge et al., 2002). Thus, even if the mean hippocampal volume was reduced across the perinatal period in our participants, the absolute hippocampal volume may still be within the same range as the healthy controls. Intriguingly, hippocampal volume postpartum was associated with subclinical depressive symptoms, but in the opposite direction of what we would expect from studies in clinically depressed patients (Schmaal et al., 2016). Notably, we do not have any longitudinal data and thus do not know perinatal the within-subject hippocampal changes. However, if healthy women normally experience a decrease

in hippocampal volume, a large volume may reflect a less healthy transition, compared to a smaller volume. Animal studies support that the brain undergoes functional changes to optimize it for the demands of the postpartum period and that this may in part depend on the hippocampal plasticity in the perinatal period (Been et al., 2021; Pawluski et al., 2021). A high postpartum hippocampal volume may therefore reflect a maladaptive plasticity across pregnancy and thus increase the susceptibility to postpartum mental distress. Intriguingly, DNA methylation markers associated with postpartum depression map on to genes that code for hippocampal plasticity, perhaps reflecting a maladaptive hippocampal plasticity.

Our data indicate that this may be related to altered 5-HT4R availability. Interaction analyses showed that in women with high brain 5-HT4R, the magnitude of the estradiol decline was inversely associated with hippocampal volume, while in women with lower brain 5-HT4R the magnitude of the decline was positively associated with hippocampal volume. Thus, a small estradiol decrease in women with high 5-HT4R or a large estradiol decrease in women with lower 5-HT4R, may both lead to maladaptive hippocampal plasticity. This is intriguing since 5-HT4R may mediate some of its positive effects through increased neuroplasticity in hippocampus (Hagena and Manahan-Vaughan, 2017). Our data suggests that sex-hormones may also be involved in such processes. This may map on to the genomic estradiol sensitivity markers associated with risk for PND, as they also affect genes that code for the serotonin system and hippocampal volume (Guintivano et al., 2014; Mehta et al., 2019). Although speculative, it is possible that such mechanisms also play a role in relation to oral contraceptive use, which suppresses endogenous sex-hormones and has been associated with increased hippocampal volume, reduced 5-HT4R brain binding and depressive symptoms (Larsen et al., 2020; Pletzer et al., 2019, 2010; Skovlund et al., 2016).

Discussion across the studies

As is evident from the introduction, our hypotheses for the perinatal transition study were largely based on what we learned from the pharmacological sex-hormone manipulation model. Specifically, we expected hippocampal volume changes in the sex-hormone manipulation model to translate to the postpartum state. As we did not have any longitudinal data, we expected that the more dramatic perinatal hormone changes, would lead to reduced hippocampal volume compared

to . It turned out that healthy postpartum women maintained a hippocampal volume similar to healthy controls. Further, we were not able to demonstrate that GnRHa induced hippocampal volume changes that were significantly different from natural hippocampal volume fluctuations in women who were not hormonally manipulated either. Thus, in both cases, hippocampal volume remained within the normal range, suggesting that the healthy female brain adjusts well to hormone changes. Mental distress mapped on to high hippocampal volume in postpartum women, but was not linked to volume changes in the GnRHa group. Thus, the mechanisms that regulate mental health may be substantially different during the two types of hormone transitions. In line with this, a large perinatal decrease in estradiol promoted mental health, while a large decrease in response to GnRHa resulted in more depressive symptoms (Frokjaer et al., 2015). As indicated throughout the thesis and noted by others (Rehbein et al., 2021), the direction of estrogen effects on neurobiological markers is not conclusive. Taken together, our results indicate that the length and magnitude of an estradiol fluctuation may have substantial consequences for emotional responses to sex-hormone changes and related neurobiological changes. That is, the months of constantly increasing estrogens in pregnancy and the abrupt decrease may have completely different effects on the brain than the short-term, small magnitude fluctuations in the sex-hormone manipulation model. There are some data to support that long or short-term exposure to estrogens may have differential effects, e.g. on SERT regulation (Deecher et al., 2008). Similarly, dynamic fluctuations in endogenous estrogens and static concentrations of exogenous estrogens may have substantially different effects on the brain, as seen for 5-HT4R (Larsen et al., 2020). An interesting notion is that both in the sex-hormone manipulation study and the perinatal study, the association between hippocampal volume and estradiol changes were only evident when taking serotonergic markers in to account. This suggests that estradiol and serotonergic signaling has complimentary effects on hippocampal plasticity in promoting mental health during hormone transitions.

The study population in Study 2 and 3 largely overlap, as the participants for the neuroimaging study were recruited from the Study 2. A large decrease in estradiol was both associated with fewer distress symptoms in Study 2 and interacted with a high 5-HT4R in promoting a small hippocampal volume, which in turn mapped on to fewer mental distress symptoms. Thus, it is

possible that a part of the beneficial effect of a large decrease in estradiol was linked to 5-HT4R availability and hippocampal plasticity. Intriguingly, this "protective effect" of a large estradiol decrease was most pronounced for women with maternal experience. Animal data suggests that parity and maternal experience may increase hippocampal plasticity, including sensitivity to estrogens, long-term and affect hippocampal plasticity in subsequent pregnancies (Barha et al., 2015; Barha and Galea, 2011; Eid et al., 2019; Pawluski and Galea, 2006). It is possible that the effect of previous pregnancies on hippocampal plasticity played a role in this, perhaps involving 5-HT4R. Both studies suggests that adaptability may key in healthy perinatal transitions, either in terms of an appropriate serotonergic response to high levels of estrogens in pregnancy or in plastic remodeling of the brain. This aligns well with reports of high hippocampal plasticity and plastic changes in the dorsal raphe nuclei during the perinatal period in animal models, which both seems to support healthy maternal behavior (Holschbach and Lonstein, 2017; Medina and Workman, 2020; Pawluski et al., 2021).

Methodological considerations

Across all three studies, limited reproducibility to clinical cohorts is an issue. In both cases, this is inherent to the study design, selected to ease the interpretation of post-transition distress symptoms. However, this excludes a number of potential risk factors that may interact with the proposed risk mechanisms to promote clinical levels of depression. This is perhaps even more pronounced in the perinatal study, especially the imaging part, as the demanding study program probably led to selection bias.

For both imaging studies, we may well have been underpowered. The sex-hormone model was designed for PET neuroimaging purposes, and not hippocampal volume assessment. Power calculations for the PET analyses in the perinatal study were based on power analyses from non-postpartum cohorts (Marner et al., 2010) and may not map well on to the postpartum. Further, hippocampal volume changes in healthy individuals are likely to be discrete thus vulnerable to measurement noise. For the perinatal imaging subgroup, we were unfortunately not able to include age-matched controls (historic controls). This seemed of minor importance for the hippocampal volume differences, but age-adjusting affected the group difference for 5-HT4R to some extent. In both cases, younger controls may have had higher 5-HT4R or hippocampal volume

than age-matched controls, as both decrease with age (Madsen et al., 2011; Marner et al., 2010; Nobis et al., 2019). Thus, we are less likely to have missed a significantly reduced postpartum hippocampal volume or 5-HT4R brain binding, than a significantly increased one.
In the perinatal study, drop-outs and missing data was an issue which we tried to compensate for by dividing the models for 5-HIAA and estradiol. Supplementary analyses, detailed in supplementary materials for Manuscript 2, indicated that we may have underestimated the statistical significance for the 5-HIAA association on this account. Furthermore, it is possible that some of the women we lost contact with or who did not complete their postpartum EPDS may have had more distress symptoms. As detailed in supplementary materials for Manuscript 2, many of the women we lost contact to had more sleep disturbances in pregnancy, which is a risk factor for depression (Emamian et al., 2019).

Conclusion and perspectives

This thesis details how two different sex-hormone transition models may map on to mental distress and relevant neurobiological mechanisms. Our data indicate that while the short-term estradiol changes in the sex-hormone manipulation model map on to hippocampal volume, when adjusted for SERT at baseline, they do not seem to be associated with the emergence of subclinical depressive symptoms. In line with this, the magnitude of the volume changes in response to GnRHa are similar to natural, non-manipulated (placebo) fluctuations in hippocampal volume. In relation to pregnancy and birth, our results show that the large perinatal estradiol changes, i.e., returning to pre-pregnancy levels, may protect mental health in the majority of women, pointing towards an altered estrogen sensitivity in women who develop a depression. Conversely, serotonergic markers (5-HIAA) that may map on to SERT expression in pregnancy, seem to induce postpartum mental distress in healthy women. Further, subclinical levels of postpartum mental distress seemed to be associated with a greater hippocampal volume, which we speculate may reflect a maladaptive hippocampal plasticity across peripartum. Our results implicate 5-HT4R binding in interaction with estradiol changes in hippocampal plasticity during healthy perinatal transitions, that may be involved in resilience and thus relevant in depression, too. Taken together our data suggest that estradiol changes and serotonergic signaling may have complimentary roles in hippocampal plasticity during sex-hormone transitions. Our results highlight that the length and

magnitude of an estradiol fluctuation may be key in how it maps on to neurobiological changes and potential depressive symptoms. Finally, our results show that the healthy brain adapts well to sex-hormone transitions.

Overall, our findings provide novel insights to mechanisms that are important in healthy sex-hormone transitions. While we cannot extrapolate our results to clinical cohorts, our results may reflect subclinical manifestations of relevant risk mechanisms. However, these associations should be replicated in studies with larger sample size, preferably in high-risk samples or in samples with a wider range of mental distress symptoms, to determine their clinical relevance. Especially the relation between estradiol, 5-HT4R and hippocampal plasticity points towards a mechanism that may be relevant for depressive responses during the perinatal transition phase. However, particularly rodent studies of 5-HT4R contributions to hippocampal plasticity, in relation to sex-hormone transitions and the peripartum, are warranted. Further, human studies of grey matter volume brain changes in women with PND are needed to determine if and how perinatal hippocampal volume changes are involved in PND. It may be relevant to evaluate if such changes map on to estrogen dependent genomic risk markers for PND (Guintivano et al., 2014). Ideally, such analyses should be supplemented with *in vivo* imaging of potential plasticity proxies, not just 5-HT4R brain binding, but also e.g. markers for synaptic density (Finnema et al., 2016). Similarly, further elucidation of the role of SERT availability in mental distress and hippocampal plasticity during sex-hormone transitions may also be relevant. Finally, the role of estrogen sensitivity in depressive responses during hormone transitions may still need further evaluation. Future studies in high risk populations such as Høgh et al. (2021), may help understand sex-hormone involvement in PND better.

In addition to evaluating the proposed mechanisms in other populations, it may also be relevant to explore how they relate to other neurobiological markers. We have the capacity to do this within our cohort of healthy pregnant and postpartum women, as data collected in this cohort extend well beyond what has been detailed under Study 2 and 3. Thus, future studies by our group or others may reveal if the observed associations map on to e.g., functional MRI parameters, stress-biology, inflammatory markers, or synaptic density.

In summary, we here show that estradiol changes and serotonergic signaling in concert play an important role in hippocampal plasticity during sex-hormone transitions, but that the length and magnitude of an estradiol fluctuation may be key in how it maps on to neurobiological changes and depressive symptoms. Our data points towards potential risk mechanisms for depressive responses during sex-hormone transitions, but further studies, especially in high-risk samples or depressed populations, are warranted to elucidate their clinical relevance.

References

Aasheim, V., Waldenström, U., Hjelmstedt, A., Rasmussen, S., Pettersson, H., Schytt, E., 2012. Associations between advanced maternal age and psychological distress in primiparous women, from early pregnancy to 18 months postpartum. BJOG An Int. J. Obstet. Gynaecol. 119, 1108–1116. https://doi.org/10.1111/J.1471-0528.2012.03411.X

Albert, K., Hiscox, J., Boyd, B., Dumas, J., Taylor, W., Newhouse, P., 2017. Estrogen enhances hippocampal gray-matter volume in young and older postmenopausal women: a prospective dose-response study. Neurobiol. Aging 56, 1–6. https://doi.org/10.1016/j.neurobiolaging.2017.03.033

Alenina, N., Klempin, F., 2015. The role of serotonin in adult hippocampal neurogenesis. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2014.07.038

Altemus, M., Fong, J., Yang, R., Damast, S., Luine, V., Ferguson, D., 2004. Changes in cerebrospinal fluid neurochemistry during pregnancy. Biol. Psychiatry 56, 386–392. https://doi.org/10.1016/j.biopsych.2004.06.002

American Psychiatric Association. DSM-5 Task Force., 2013. Diagnostic and statistical manual of mental disorders : DSM-5. American Psychiatric Association.

Amin, Z., Gueorguieva, R., Cappiello, A., Czarkowski, K.A., Stiklus, S., Anderson, G.M., Naftolin, F., Epperson, C.N., 2006. Estradiol and tryptophan depletion interact to modulate cognition in menopausal women. Neuropsychopharmacology 31, 2489–2497. https://doi.org/10.1038/sj.npp.1301114

Baka, J., Csakvari, E., Huzian, O., Dobos, N., Siklos, L., Leranth, C., MacLusky, N.J., Duman, R.S., Hajszan, T., 2017. Stress induces equivalent remodeling of hippocampal spine synapses in a simulated postpartum environment and in a female rat model of major depression. Neuroscience 343, 384–397. https://doi.org/10.1016/j.neuroscience.2016.12.021

Barha, C.K., Galea, L.A.M., 2011. Motherhood alters the cellular response to estrogens in the hippocampus later in life. Neurobiol. Aging 32, 2091–2095. https://doi.org/10.1016/j.neurobiolaging.2009.12.004

Barha, C.K., Lieblich, S.E., Chow, C., Galea, L.A.M., 2015. Multiparity-induced enhancement of hippocampal neurogenesis and spatial memory depends on ovarian hormone status in middle age. Neurobiol. Aging 36, 2391–2405. https://doi.org/10.1016/j.neurobiolaging.2015.04.007

Barth, C., Steele, C.J., Mueller, K., Rekkas, V.P., Arélin, K., Pampel, A., Burmann, I., Kratzsch, J., Villringer, A., Sacher, J., 2016. In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. Sci. Rep. 6, 32833. https://doi.org/10.1038/srep32833

Barth, C., Villringer, A., Sacher, J., 2015. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. Front. Neurosci. 9. https://doi.org/10.3389/fnins.2015.00037

Bartolomucci, A., Carola, V., Pascucci, T., Puglisi-Allegra, S., Cabib, S., Lesch, K.P., Parmigiani, S., Palanza, P., Gross, C., 2010. Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. DMM Dis. Model. Mech. 3, 459–470. https://doi.org/10.1242/dmm.004614

Bech, P., Rasmussen, N.A., Olsen, L.R., Noerholm, V., Abildgaard, W., 2001. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. J. Affect. Disord. 66, 159–164. https://doi.org/10.1016/S0165-0327(00)00309-8

Bech, P., Timmerby, N., Martiny, K., Lunde, M., Soendergaard, S., 2015. Psychometric evaluation of the Major Depression Inventory (MDI) as depression severity scale using the LEAD (Longitudinal Expert Assessment of All Data) as index of validity. BMC Psychiatry 15. https://doi.org/10.1186/s12888-015-0529-3

Beck, C.T., 2001. Predictors of postpartum depression: An update. Nurs. Res. 50, 275–285. https://doi.org/10.1097/00006199-200109000-00004

Been, L.E., Sheppard, P.A.S., Galea, L.A.M., Glasper, E.R., 2021. Hormones and neuroplasticity: A lifetime of adaptive responses. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2021.11.029

Beliveau, V., Ganz, M., Feng, L., Ozenne, B., Højgaard, L., Fisher, P.M., Svarer, C., Greve, D.N., Knudsen, G.M., 2017. A high-resolution in vivo atlas of the human brain's serotonin system. J. Neurosci. 37, 120–128. https://doi.org/10.1523/JNEUROSCI.2830-16.2016

Bethea, C.L., Lu, N.Z., Gundlah, C., Streicher, J.M., 2002. Diverse actions of ovarian steroids in the serotonin neural system. Front. Neuroendocrinol. https://doi.org/10.1006/frne.2001.0225

Blackmore, E.R., Gustafsson, H., Gilchrist, M., Wyman, C., O'Connor, T.G., 2016. Pregnancy-related anxiety: Evidence of distinct clinical significance from a prospective longitudinal study. J.

Affect. Disord. 197, 251–258. https://doi.org/10.1016/j.jad.2016.03.008

Bloch, M., Schmidt, P.J., Danaceau, M., Murphy, J., Nieman, L., Rubinow, D.R., 2000. Effects of gonadal steroids in women with a history of postpartum depression. Am. J. Psychiatry 157, 924–930. https://doi.org/10.1176/appi.ajp.157.6.924

Bødker, B., Hvidman, L., Weber, T., Møller, M., Andersen, B.R., Westergaard, H.B., Rosthøj, S., Sørensen, J.L., 2021a. Reduction in maternal mortality in Denmark over three decades. Dan. Med. J. 68, 2210143.

Bødker, B., Hvidman, L., Weber, T., Møller, M., Sørensen, J.L., 2021b. An audit of 70 maternal deaths. Dan. Med. J. 68, 2210144.

Borrow, A.P., Cameron, N.M., 2014. Estrogenic mediation of serotonergic and neurotrophic systems: Implications for female mood disorders. Prog. Neuro-Psychopharmacology Biol. Psychiatry 54, 13–25. https://doi.org/10.1016/j.pnpbp.2014.05.009

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213. https://doi.org/10.1016/0165-1781(89)90047-4

Carpenter, L.L., Anderson, G.M., Siniscalchi, J.M., Chappell, P.B., Price, L.H., 2003. Acute changes in cerebrospinal fluid 5-HIAA following oral paroxetine challenge in healthy humans. Neuropsychopharmacology 28, 339–347. https://doi.org/10.1038/sj.npp.1300025

Chatzicharalampous, C., Rizos, D., Pliatsika, P., Leonardou, A., Hasiakos, D., Zervas, I., Alexandrou, A., Creatsa, M., Konidaris, S., Lambrinoudaki, I., 2011. Reproductive hormones and postpartum mood disturbances in Greek women. Gynecol. Endocrinol. 27, 543–550. https://doi.org/10.3109/09513590.2010.501886

Cox, J.L., Holden, J.M., Sagovsky, R., 1987. Detection of Postnatal Depression: Development of the 10-item Edinburgh Postnatal Depression scale. Br. J. Psychiatry 150, 782–786. https://doi.org/10.1192/bjp.150.6.782

De Bellis, M.D., Geracioti, T.D., Altemus, M., Kling, M.A., 1993. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. Biol. Psychiatry 33, 636–641. https://doi.org/10.1016/0006-3223(93)90103-K

de Cates, A.N., Wright, L.C., Martens, M.A.G., Gibson, D., Türkmen, C., Filippini, N., Cowen, P.J., Harmer, C.J., Murphy, S.E., 2021. Déjà-vu? Neural and behavioural effects of the 5-HT4

receptor agonist, prucalopride, in a hippocampal-dependent memory task. Transl. Psychiatry 11, 497. https://doi.org/10.1038/s41398-021-01568-4

de Rezende, M.G., Garcia-Leal, C., Silva de Sá, M.F., Cavalli, R. de C., Del-Ben, C.M., 2019. Withdrawal of plasma estradiol is associated with increased anxiety reported by women in the first 12 hours after delivery. Psychoneuroendocrinology 101, 67–71. https://doi.org/10.1016/j.psyneuen.2018.11.006

Deecher, D., Andree, T.H., Sloan, D., Schechter, L.E., 2008. From menarche to menopause: Exploring the underlying biology of depression in women experiencing hormonal changes. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2007.10.006

Del Barrio, V., 2016. Diagnostic and statistical manual of mental disorders. Curated Ref. Collect. Neurosci. Biobehav. Psychol. 1–943. https://doi.org/10.1016/B978-0-12-809324-5.05530-9

Deligiannidis, K.M., Sikoglu, E.M., Shaffer, S.A., Frederick, B., Svenson, A.E., Kopoyan, A., Kosma, C.A., Rothschild, A.J., Moore, C.M., 2013. GABAergic neuroactive steroids and resting-state functional connectivity in postpartum depression: A preliminary study. J. Psychiatr. Res. 47, 816–828. https://doi.org/10.1016/j.jpsychires.2013.02.010

Denoth-Lippuner, A., Jessberger, S., 2021. Formation and integration of new neurons in the adult hippocampus. Nat. Rev. Neurosci. https://doi.org/10.1038/s41583-021-00433-z

Eid, R.S., Chaiton, J.A., Lieblich, S.E., Bodnar, T.S., Weinberg, J., Galea, L.A.M., 2019. Early and late effects of maternal experience on hippocampal neurogenesis, microglia, and the circulating cytokine milieu. Neurobiol. Aging 78, 1–17. https://doi.org/10.1016/j.neurobiolaging.2019.01.021

Emamian, F., Khazaie, H., Okun, M.L., Tahmasian, M., Sepehry, A.A., 2019. Link between insomnia and perinatal depressive symptoms: A meta-analysis. J. Sleep Res. 28, e12858. https://doi.org/10.1111/jsr.12858

Eriksson, E., Alling, C., Andersch, B., Andersson, K., Berggren, U., 1994. Cerebrospinal fluid levels of monoamine metabolites a preliminary study of their relation to menstrual cycle phase, sex steroids, and pituitary hormones in healthy women and in women with premenstrual syndrome. Neuropsychopharmacology 11, 201–213. https://doi.org/10.1038/sj.npp.1380107

Evans, J., Melotti, R., Heron, J., Ramchandani, P., Wiles, N., Murray, L., Stein, A., 2012. The timing of maternal depressive symptoms and child cognitive development: A longitudinal study. J.

Child Psychol. Psychiatry Allied Discip. 53, 632–640. https://doi.org/10.1111/j.1469-7610.2011.02513.x

Fan, F., Zou, Y., Ma, A., Yue, Y., Mao, W., Ma, X., 2009. Hormonal changes and somatopsychologic manifestations in the first trimester of pregnancy and post partum. Int. J. Gynecol. Obstet. 105, 46–49. https://doi.org/10.1016/j.ijgo.2008.12.001

Finnema, S.J., Nabulsi, N.B., Eid, T., Detyniecki, K., Lin, S.F., Chen, M.K., Dhaher, R., Matuskey, D., Baum, E., Holden, D., Spencer, D.D., Mercier, J., Hannestad, J., Huang, Y., Carson, R.E., 2016. Imaging synaptic density in the living human brain. Sci. Transl. Med. 8. https://doi.org/10.1126/SCITRANSLMED.AAF6667

Fischl, B., 2012. FreeSurfer. Neuroimage. https://doi.org/10.1016/j.neuroimage.2012.01.021

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., Van Der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X

Fisher, P.M., Holst, K.K., Mc Mahon, B., Haahr, M.E., Madsen, K., Gillings, N., Baaré, W.F., Jensen, P.S., Knudsen, G.M., 2012. 5-HTTLPR status predictive of neocortical 5-HT4binding assessed with [11C]SB207145 PET in humans. Neuroimage 62, 130–136. https://doi.org/10.1016/j.neuroimage.2012.05.013

Fisher, P.M.D., Larsen, C.B., Beliveau, V., Henningsson, S., Pinborg, A., Holst, K.K., Jensen, P.S., Svarer, C., Siebner, H.R., Knudsen, G.M., Frokjaer, V.G., 2017. Pharmacologically Induced Sex Hormone Fluctuation Effects on Resting-State Functional Connectivity in a Risk Model for Depression: A Randomized Trial. Neuropsychopharmacology 42, 446–453. https://doi.org/10.1038/npp.2016.208

Fraser, M.A., Shaw, M.E., Cherbuin, N., 2015. A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. Neuroimage 112, 364–374. https://doi.org/10.1016/j.neuroimage.2015.03.035

Frederiksen, H., Johannsen, T.H., Andersen, S.E., Albrethsen, J., Landersoe, S.K., Petersen, J.H., Andersen, A.N., Vestergaard, E.T., Schorring, M.E., Linneberg, A., Main, K.M., Andersson, A.M., Juul, A., 2020. Sex-specific Estrogen Levels and Reference Intervals from Infancy to Late Adulthood Determined by LC-MS/MS. J. Clin. Endocrinol. Metab. 105, 754–768.

https://doi.org/10.1210/clinem/dgz196

Freeman, E.W., Sammel, M.D., Lin, H., Nelson, D.B., 2006. Associations of hormones and menopausal status with depressed mood in women with no history of depression. Arch. Gen. Psychiatry 63, 375–382. https://doi.org/10.1001/archpsyc.63.4.375

Frokjaer, V.G., 2020. Pharmacological sex hormone manipulation as a risk model for depression. J. Neurosci. Res. https://doi.org/10.1002/jnr.24632

Frokjaer, V.G., Erritzoe, D., Juul, A., Nielsen, F.Å., Holst, K., Svarer, C., Madsen, J., Paulson, O.B., Knudsen, G.M., 2010. Endogenous plasma estradiol in healthy men is positively correlated with cerebral cortical serotonin 2A receptor binding. Psychoneuroendocrinology 35, 1311–1320. https://doi.org/10.1016/j.psyneuen.2010.03.002

Frokjaer, V.G., Pinborg, A., Holst, K.K., Overgaard, A., Henningsson, S., Heede, M., Larsen, E.C., Jensen, P.S., Agn, M., Nielsen, A.P., Stenbæk, D.S., Da Cunha-Bang, S., Lehel, S., Siebner, H.R., Mikkelsen, J.D., Svarer, C., Knudsen, G.M., 2015. Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: A positron emission tomography study. Biol. Psychiatry 78, 534–543. https://doi.org/10.1016/j.biopsych.2015.04.015

Galea, L.A.M., Spritzer, M.D., Barker, J.M., Pawluski, J.L., 2006. Gonadal hormone modulation of hippocampal neurogenesis in the adult. Hippocampus 16, 225–232. https://doi.org/10.1002/hipo.20154

Galea, L.A.M.M., Leuner, B., Slattery, D.A., 2014. Hippocampal Plasticity during the Peripartum Period: Influence of Sex Steroids, Stress and Ageing. J. Neuroendocrinol. 26, 641–648. https://doi.org/10.1111/jne.12177

García-Blanco, A., Monferrer, A., Grimaldos, J., Hervás, D., Balanzá-Martínez, V., Diago, V., Vento, M., Cháfer-Pericás, C., 2017. A preliminary study to assess the impact of maternal age on stress-related variables in healthy nulliparous women. Psychoneuroendocrinology 78, 97–104. https://doi.org/10.1016/j.psyneuen.2017.01.018

Gavin, N.I., Gaynes, B.N., Lohr, K.N., Meltzer-Brody, S., Gartlehner, G., Swinson, T., 2005. Perinatal depression: A systematic review of prevalence and incidence. Obstet. Gynecol. 106, 1071–1083. https://doi.org/10.1097/01.AOG.0000183597.31630.db

Glover, V., 2014. Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. Best Pract. Res. Clin. Obstet. Gynaecol. 28, 25–35.

https://doi.org/10.1016/j.bpobgyn.2013.08.017

Goodman, S.H., Rouse, M.H., Connell, A.M., Broth, M.R., Hall, C.M., Heyward, D., 2011. Maternal Depression and Child Psychopathology: A Meta-Analytic Review. Clin. Child Fam. Psychol. Rev. https://doi.org/10.1007/s10567-010-0080-1

Gould, E., Tanapat, P., Rydel, T., Hastings, N., 2000. Regulation of hippocampal neurogenesis in adulthood. Biol. Psychiatry 48, 715–720. https://doi.org/10.1016/S0006-3223(00)01021-0

Grant, K.A., McMahon, C., Austin, M.P., 2008. Maternal anxiety during the transition to parenthood: A prospective study. J. Affect. Disord. 108, 101–111. https://doi.org/10.1016/j.jad.2007.10.002

Guintivano, J., Arad, M., Gould, T.D., Payne, J.L., Kaminsky, Z.A., 2014. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. Mol. Psychiatry 19, 560–567. https://doi.org/10.1038/mp.2013.62

Guintivano, J., Manuck, T., Meltzer-Brody, S., 2018. Predictors of Postpartum Depression: A Comprehensive Review of the Last Decade of Evidence. Clin. Obstet. Gynecol. 61, 591–603. https://doi.org/10.1097/GRF.0000000000000368

Haahr, M.E., Fisher, P., Holst, K., Madsen, K., Jensen, C.G., Marner, L., Lehel, S., Baaré, W., Knudsen, G., Hasselbalch, S., 2013. The 5-HT4 receptor levels in hippocampus correlates inversely with memory test performance in humans. Hum. Brain Mapp. 34, 3066–3074. https://doi.org/10.1002/hbm.22123

Haahr, M.E., Fisher, P.M., Jensen, C.G., Frokjaer, V.G., McMahon, B., Madsen, K., Baaré, W.F.C., Lehel, S., Norremolle, A., Rabiner, E.A., Knudsen, G.M., 2014. Central 5-HT4 receptor binding as biomarker of serotonergic tonus in humans: A [11C]SB207145 PET study. Mol. Psychiatry 19, 427–432. https://doi.org/10.1038/mp.2013.147

Hagena, H., Manahan-Vaughan, D., 2017. The serotonergic 5-HT4 receptor: A unique modulator of hippocampal synaptic information processing and cognition. Neurobiol. Learn. Mem. 138, 145–153. https://doi.org/10.1016/j.nlm.2016.06.014

HAMILTON, M., 1967. Development of a Rating Scale for Primary Depressive Illness. Br. J. Soc. Clin. Psychol. 6, 278–296. https://doi.org/10.1111/j.2044-8260.1967.tb00530.x

Hampson, E., Phillips, S.D., Duff-Canning, S.J., Evans, K.L., Merrill, M., Pinsonneault, J.K., Sadée, W., Soares, C.N., Steiner, M., 2015. Working memory in pregnant women: Relation to estrogen

and antepartum depression. Horm. Behav. 74, 218–227. https://doi.org/10.1016/j.yhbeh.2015.07.006

Harris, B., Lovett, L., Newcombe, R.G., Read, G.F., Walker, R., Riad-Fahmy, D., 1994. Maternity blues and major endocrine changes: Cardiff puerperal mood and hormone study II. Bmj 308, 949. https://doi.org/10.1136/bmj.308.6934.949

Heidrich, A., Schleyer, M., Spingler, H., Albert, P., Knoche, M., Fritze, J., Lanczik, M., 1994. Postpartum blues: Relationship between not-protein bound steroid hormones in plasma and postpartum mood changes. J. Affect. Disord. 30, 93–98. https://doi.org/10.1016/0165-0327(94)90036-1

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation of human serotonin transporter gene expression. J. Neurochem. 66, 2621–2624. https://doi.org/10.1046/j.1471-4159.1996.66062621.x

Henningsson, S., Madsen, K.H., Pinborg, A., Heede, M., Knudsen, G.M., Siebner, H.R., Frokjaer, V.G., 2015. Role of emotional processing in depressive responses to sex-hormone manipulation: A pharmacological fMRI study. Transl. Psychiatry 5, e688–e688. https://doi.org/10.1038/tp.2015.184

Heron, J., O'Connor, T.G., Evans, J., Golding, J., Glover, V., 2004. The course of anxiety and depression through pregnancy and the postpartum in a community sample. J. Affect. Disord. 80, 65–73. https://doi.org/10.1016/j.jad.2003.08.004

Heurling, K., Leuzy, A., Jonasson, M., Frick, A., Zimmer, E.R., Nordberg, A., Lubberink, M., 2017. Quantitative positron emission tomography in brain research. Brain Res. 1670, 220–234. https://doi.org/10.1016/j.brainres.2017.06.022

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J.C., Tobeña, A., Desco, M., Crone, E.A., Ballesteros, A., Carmona, S., Vilarroya, O., 2017. Pregnancy leads to long-lasting changes in human brain structure. Nat. Neurosci. 20, 287–296. https://doi.org/10.1038/nn.4458

Høgh, S., Hegaard, H.K., Renault, K.M., Cvetanovska, E., Kjærbye-Thygesen, A., Juul, A., Borgsted, C., Bjertrup, A.J., Miskowiak, K.W., Væver, M.S., Stenbæk, D.S., Dam, V.H., Binder, E., Ozenne, B., Mehta, D., Frokjaer, V.G., 2021. Short-term oestrogen as a strategy to prevent postpartum depression in high-risk women: protocol for the double-blind, randomised, placebo-

controlled MAMA clinical trial. BMJ Open 11, e052922. https://doi.org/10.1136/bmjopen-2021-052922

Holschbach, M.A., Lonstein, J.S., 2017. Motherhood and infant contact regulate neuroplasticity in the serotonergic midbrain dorsal raphe. Psychoneuroendocrinology 76, 97–106. https://doi.org/10.1016/j.psyneuen.2016.10.023

Howard, L.M., Molyneaux, E., Dennis, C.L., Rochat, T., Stein, A., Milgrom, J., 2014. Non-psychotic mental disorders in the perinatal period. Lancet 384, 1775–1788. https://doi.org/10.1016/S0140-6736(14)61276-9

Jennings, K.A., Licht, C.L., Bruce, A., Lesch, K.P., Knudsen, G.M., Sharp, T., 2012. Genetic variation in 5-hydroxytryptamine transporter expression causes adaptive changes in 5-HT4 receptor levels. Int. J. Neuropsychopharmacol. 15, 1099–1107. https://doi.org/10.1017/S1461145711001258

Kalbitzer, J., Erritzoe, D., Holst, K.K., Nielsen, F.Å., Marner, L., Lehel, S., Arentzen, T., Jernigan, T.L., Knudsen, G.M., 2010. Seasonal Changes in Brain Serotonin Transporter Binding in Short Serotonin Transporter Linked Polymorphic Region-Allele Carriers but Not in Long-Allele Homozygotes. Biol. Psychiatry 67, 1033–1039. https://doi.org/10.1016/j.biopsych.2009.11.027

Kim, P., Leckman, J.F., Mayes, L.C., Feldman, R., Wang, X., Swain, J.E., 2010. The plasticity of human maternal brain: Longitudinal changes in brain anatomy during the early postpartum period. Behav. Neurosci. 124, 695–700. https://doi.org/10.1037/a0020884

Klier, C.M., Muzik, M., Dervic, K., Mossaheb, N., Benesch, T., Ulm, B., Zeller, M., 2007. The role of estrogen and progesterone in depression after birth. J. Psychiatr. Res. 41, 273–279. https://doi.org/10.1016/j.jpsychires.2006.09.002

Knudsen, G.M., Jensen, P.S., Erritzoe, D., Baaré, W.F.C., Ettrup, A., Fisher, P.M., Gillings, N., Hansen, H.D., Hansen, L.K., Hasselbalch, S.G., Henningsson, S., Herth, M.M., Holst, K.K., Iversen, P., Kessing, L. V., Macoveanu, J., Madsen, K.S., Mortensen, E.L., Nielsen, F.Å., Paulson, O.B., Siebner, H.R., Stenbæk, D.S., Svarer, C., Jernigan, T.L., Strother, S.C., Frokjaer, V.G., 2016. The Center for Integrated Molecular Brain Imaging (Cimbi) database. Neuroimage 124, 1213–1219. https://doi.org/10.1016/j.neuroimage.2015.04.025

Koehler-Forsberg, K., Dam, V., Joergensen, A., Landman, E., Larsen, S.V., Poulsen, A.S., Teng-Ip, C.,

Joergensen, M.B., Frokjaer, V.G., Knudsen, G.M., 2019. P.352 Patients with major depressive disorder have lower cerebral serotonin 4 receptor binding than healthy controls. Eur. Neuropsychopharmacol. 29, S251–S252. https://doi.org/10.1016/j.euroneuro.2019.09.370

Kraus, C., Castrén, E., Kasper, S., Lanzenberger, R., 2017. Serotonin and neuroplasticity – Links between molecular, functional and structural pathophysiology in depression. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2017.03.007

Kuehner, C., 2017. Why is depression more common among women than among men? The Lancet Psychiatry 4, 146–158. https://doi.org/10.1016/S2215-0366(16)30263-2

Kuijper, E.A.M., Ket, J.C.F., Caanen, M.R., Lambalk, C.B., 2013. Reproductive hormone concentrations in pregnancy and neonates: A systematic review. Reprod. Biomed. Online. https://doi.org/10.1016/j.rbmo.2013.03.009

Lammertsma, A.A., 2019. Essentials of Quantitative Imaging with PET. Nucl. Med. Textb. 219–233. https://doi.org/10.1007/978-3-319-95564-3_10

Lammertsma, A.A., Hume, S.P., 1996. Simplified reference tissue model for PET receptor studies. Neuroimage 4, 153–158. https://doi.org/10.1006/nimg.1996.0066

Larsen, S. V., Köhler-Forsberg, K., Dam, V.H., Poulsen, A.S., Svarer, C., Jensen, P.S., Knudsen, G.M., Fisher, P.M., Ozenne, B., Frokjaer, V.G., 2020. Oral contraceptives and the serotonin 4 receptor: a molecular brain imaging study in healthy women. Acta Psychiatr. Scand. 142, 294–306. https://doi.org/10.1111/acps.13211

Licht, Cecilie L., Kirkegaard, L., Zueger, M., Chourbaji, S., Gass, P., Aznar, S., Knudsen, G.M., 2010. Changes in 5-HT4 receptor and 5-HT transporter binding in olfactory bulbectomized and glucocorticoid receptor heterozygous mice. Neurochem. Int. 56, 603–610. https://doi.org/10.1016/j.neuint.2010.01.003

Licht, Cecilie Löe, Knudsen, G.M., Sharp, T., 2010. Effects of the 5-HT4 receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. Neurosci. Lett. 476, 58–61. https://doi.org/10.1016/j.neulet.2010.04.002

Lisofsky, N., Gallinat, J., Lindenberger, U., Kühn, S., 2019. Postpartal Neural Plasticity of the Maternal Brain: Early Renormalization of Pregnancy-Related Decreases? Neurosignals. 27, 12–24. https://doi.org/10.33594/000000105

Lisofsky, N., Mårtensson, J., Eckert, A., Lindenberger, U., Gallinat, J., Kühn, S., 2015. Hippocampal

volume and functional connectivity changes during the female menstrual cycle. Neuroimage 118, 154–162. https://doi.org/10.1016/j.neuroimage.2015.06.012

Lokuge, S., Frey, B.N., Foster, J.A., Soares, C.N., Steiner, M., 2011. Depression in women: Windows of vulnerability and new insights into the link between estrogen and serotonin. J. Clin. Psychiatry 72, e1563–e1569. https://doi.org/10.4088/JCP.11com07089

Lonstein, J.S., 2019. The dynamic serotonin system of the maternal brain. Arch. Womens. Ment. Health 22, 237–243. https://doi.org/10.1007/s00737-018-0887-1

Lord, C., Buss, C., Lupien, S.J., Pruessner, J.C., 2008. Hippocampal volumes are larger in postmenopausal women using estrogen therapy compared to past users, never users and men: A possible window of opportunity effect. Neurobiol. Aging 29, 95–101. https://doi.org/10.1016/j.neurobiolaging.2006.09.001

Lu, N.Z., Eshleman, A.J., Janowsky, A., Bethea, C.L., 2003. Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution, and function in female macaques. Mol. Psychiatry 8, 353–360. https://doi.org/10.1038/sj.mp.4001243

Macbeth, A.H., Scharfman, H.E., MacLusky, N.J., Gautreaux, C., Luine, V.N., 2008. Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). Horm. Behav. 54, 7–17. https://doi.org/10.1016/j.yhbeh.2007.08.011

Macoveanu, J., Henningsson, S., Pinborg, A., Jensen, P., Knudsen, G.M., Frokjaer, V.G., Siebner, H.R., 2016. Sex-Steroid Hormone Manipulation Reduces Brain Response to Reward. Neuropsychopharmacology 41, 1057–1065. https://doi.org/10.1038/npp.2015.236

Madsen, K., Haahr, M.T., Marner, L., Keller, S.H., Baaré, W.F., Svarer, C., Hasselbalch, S.G., Knudsen, G.M., 2011. Age and sex effects on 5-HT 4 receptors in the human brain: A 11 CSB207145 PET study. J. Cereb. Blood Flow Metab. 31, 1475–1481. https://doi.org/10.1038/jcbfm.2011.11

Madsen, K., Torstensen, E., Holst, K.K., Haahr, M.E., Knorr, U., Frokjaer, V.G., Brandt-Larsen, M., Iversen, P., Fisher, P.M., Knudsen, G.M., 2015. Familial risk for major depression is associated with lower striatal 5-HT4 receptor binding. Int. J. Neuropsychopharmacol. 18, 1–7. https://doi.org/10.1093/ijnp/pyu034

Marner, L., Gillings, N., Comley, R.A., Baaré, W.F.C., Rabiner, E.A., Wilson, A.A., Houle, S.,

Hasselbalch, S.G., Svarer, C., Gunn, R.N., Laruelle, M., Knudsen, G.M., 2009. Kinetic modeling of11C-SB207145 binding to 5-HT4 receptors in the human brain in vivo. J. Nucl. Med. 50, 900–908. https://doi.org/10.2967/jnumed.108.058552

Marner, L., Gillings, N., Madsen, K., Erritzoe, D., Baaré, W.F.C., Svarer, C., Hasselbalch, S.G., Knudsen, G.M., 2010. Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. Neuroimage 50, 855–861. https://doi.org/10.1016/j.neuroimage.2010.01.054

Mårtensson, B., Wägner, A., Beck, O., Brodin, K., Montero, D., Åsberg, M., 1991. Effects of clomipramine treatment on cerebrospinal fluid monoamine metabolites and platelet H-imipramine binding and serotonin uptake and concentration in major depressive disorder. Acta Psychiatr. Scand. 83, 125–133. https://doi.org/10.1111/j.1600-0447.1991.tb07377.x

Martínez-García, M., Paternina-Die, M., Barba-Müller, E., de Blas, D.M., Beumala, L., Cortizo, R., Pozzobon, C., Marcos-Vidal, L., Fernández-Pena, A., Picado, M., Belmonte-Padilla, E., Massó-Rodriguez, A., Ballesteros, A., Desco, M., Vilarroya, Ó., Hoekzema, E., Carmona, S., 2021. Do pregnancy-induced brain changes reverse? The brain of a mother six years after parturition. Brain Sci. 11, 1–14. https://doi.org/10.3390/brainsci11020168

Matthey, S., Ross-Hamid, C., 2012. Repeat testing on the Edinburgh Depression Scale and the HADS-A in pregnancy: Differentiating between transient and enduring distress. J. Affect. Disord. 141, 213–221. https://doi.org/10.1016/j.jad.2012.02.037

Mc Mahon, B., Andersen, S.B., Madsen, M.K., Hjordt, L. V., Hageman, I., Dam, H., Svarer, C., Da Cunha-Bang, S., Baare, W., Madsen, J., Hasholt, L., Holst, K., Frokjaer, V.G., Knudsen, G.M., 2016. Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder. Brain 139, 1605–1614. https://doi.org/10.1093/brain/aww043

McKinnon, M.C., Yucel, K., Nazarov, A., MacQueen, G.M., 2009. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J. Psychiatry Neurosci. 34, 41–54.

McNeilly, A.S., Glasier, A., Jonassen, J., Howie, P.W., 1982. Evidence for direct inhibition of ovarian function by prolactin. J. Reprod. Fertil. https://doi.org/10.1530/jrf.0.0650559

McRobbie, D.W., Moore, E.A., Graves, M.J., 2017. MRI from Picture to Proton. Cambridge University Press, Cambridge. https://doi.org/10.1017/9781107706958

Medina, J., Workman, J.L., 2020. Maternal experience and adult neurogenesis in mammals: Implications for maternal care, cognition, and mental health. J. Neurosci. Res. 98, 1293–1308. https://doi.org/10.1002/jnr.24311

Mehta, D., Newport, D.J., Frishman, G., Kraus, L., Rex-Haffner, M., Ritchie, J.C., Lori, A., Knight, B.T., Stagnaro, E., Ruepp, A., Stowe, Z.N., Binder, E.B., 2014. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Psychol. Med. 44, 2309–2322. https://doi.org/10.1017/S0033291713003231

Mehta, D., Rex-Haffner, M., Sondergaard, H.B., Pinborg, A., Binder, E.B., Frokjaer, V.G., 2019. Evidence for oestrogen sensitivity in perinatal depression: Pharmacological sex hormone manipulation study. Br. J. Psychiatry 215, 519–527. https://doi.org/10.1192/bjp.2018.234

Meltzer-Brody, S., Boschloo, L., Jones, I., Sullivan, P.F., Penninx, B.W., 2013. The EPDS-Lifetime: Assessment of lifetime prevalence and risk factors for perinatal depression in a large cohort of depressed women. Arch. Womens. Ment. Health 16, 465–473. https://doi.org/10.1007/s00737-013-0372-9

Meltzer-Brody, S., Maegbaek, M.L., Medland, S.E., Miller, W.C., Sullivan, P., Munk-Olsen, T., 2017. Obstetrical, pregnancy and socio-economic predictors for new-onset severe postpartum psychiatric disorders in primiparous women, in: Psychological Medicine. pp. 1427–1441. https://doi.org/10.1017/S0033291716003020

Moses-Kolko, E.L., Wisner, K.L., Price, J.C., Berga, S.L., Drevets, W.C., Hanusa, B.H., Loucks, T.L., Meltzer, C.C., 2008. Serotonin 1A receptor reductions in postpartum depression: a positron emission tomography study. Fertil. Steril. 89, 685–692. https://doi.org/10.1016/j.fertnstert.2007.03.059

Munk-Olsen, T., Jones, I., Laursen, T.M., 2014. Birth order and postpartum psychiatric disorders. Bipolar Disord. 16, 300–307. https://doi.org/10.1111/bdi.12145

Munk-Olsen, T., Laursen, T.M., Pedersen, C.B., Mors, O., Mortensen, P.B., 2006. New parents and mental disorders: A population-based register study. J. Am. Med. Assoc. 296, 2582–2589. https://doi.org/10.1001/jama.296.21.2582

Muraca, G.M., Joseph, K.S., 2014. The Association Between Maternal Age and Depression. J. Obstet. Gynaecol. Canada 36, 803–810. https://doi.org/10.1016/S1701-2163(15)30482-5

Murphy, S.E., De Cates, A.N., Gillespie, A.L., Godlewska, B.R., Scaife, J.C., Wright, L.C., Cowen, P.J.,

Harmer, C.J., 2021. Translating the promise of 5HT 4 receptor agonists for the treatment of depression. Psychol. Med. https://doi.org/10.1017/S0033291720000604

Murphy, S.E., Wright, L.C., Browning, M., Cowen, P.J., Harmer, C.J., 2019. A role for 5-HT4 receptors in human learning and memory. Psychol. Med. 50, 2722–2730. https://doi.org/10.1017/S0033291719002836

Nikisch, G., Mathé, A.A., Czernik, A., Eap, C.B., Jiménez-Vasquez, P., Brawand-Amey, M., Baumann, P., 2004. Stereoselective metabolism of citalopram in plasma and cerebrospinal fluid of depressive patients: Relationship with 5-HIAA in CSF and clinical response. J. Clin. Psychopharmacol. 24, 283–290. https://doi.org/10.1097/01.jcp.0000125680.89843.a6

Nobis, L., Manohar, S.G., Smith, S.M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C.E., Husain, M., 2019. Hippocampal volume across age: Nomograms derived from over 19,700 people in UK Biobank. NeuroImage Clin. 23, 101904. https://doi.org/10.1016/J.NICL.2019.101904

O'Hara, M.W., Schlechte, J.A., Lewis, D.A., Wright, E.J., 1991. Prospective Study of Postpartum Blues: Biologic and Psychosocial Factors. Arch. Gen. Psychiatry 48, 801–806. https://doi.org/10.1001/archpsyc.1991.01810330025004

Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V, Puri, B.K., Fusi, L., Bydder, G.M., 2002. Change in brain size during and after pregnancy: Study in healthy women and women with preeclampsia. Am. J. Neuroradiol. 23, 19–26.

Okun, M.L., Luther, J., Prather, A.A., Perel, J.M., Wisniewski, S., Wisner, K.L., 2011. Changes in sleep quality, but not hormones predict time to postpartum depression recurrence. J. Affect. Disord. 130, 378–384. https://doi.org/10.1016/j.jad.2010.07.015

Osborne, L., Clive, M., Kimmel, M., Gispen, F., Guintivano, J., Brown, T., Cox, O., Judy, J., Meilman, S., Braier, A., Beckmann, M.W., Kornhuber, J., Fasching, P.A., Goes, F., Payne, J.L., Binder, E.B., Kaminsky, Z., 2016. Replication of Epigenetic Postpartum Depression Biomarkers and Variation with Hormone Levels. Neuropsychopharmacology 41, 1648–1658. https://doi.org/10.1038/npp.2015.333

Papageorgiou, A., Denef, C., 2007. Estradiol induces expression of 5-hydroxytryptamine (5-HT) 4, 5-HT5, and 5-HT6 receptor messenger ribonucleic acid in rat anterior pituitary cell aggregates and allows prolactin release via the 5-HT4 receptor. Endocrinology 148, 1384–1395. https://doi.org/10.1210/en.2006-1198

Pawluski, J.L., Barakauskas, V.E., Galea, L.A.M., 2010. Pregnancy decreases oestrogen receptor α expression and pyknosis, but not cell proliferation or survival, in the hippocampus. J. Neuroendocrinol. 22, 248–257. https://doi.org/10.1111/j.1365-2826.2010.01960.x

Pawluski, J.L., Brummelte, S., Barha, C.K., Crozier, T.M., Galea, L.A.M., 2009. Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. Front. Neuroendocrinol. 30, 343–357. https://doi.org/10.1016/j.yfrne.2009.03.007

Pawluski, J.L., Császár, E., Savage, E., Martinez-Claros, M., Steinbusch, H.W.M., van den Hove, D., 2015. Effects of stress early in gestation on hippocampal neurogenesis and glucocorticoid receptor density in pregnant rats. Neuroscience 290, 379–388. https://doi.org/10.1016/j.neuroscience.2015.01.048

Pawluski, J.L., Galea, L.A.M., 2006. Hippocampal morphology is differentially affected by reproductive experience in the mother. J. Neurobiol. 66, 71–81. https://doi.org/10.1002/neu.20194

Pawluski, J.L., Hoekzema, E., Leuner, B., Lonstein, J.S., 2021. Less Can Be More: Fine Tuning the Maternal Brain. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2021.11.045

Pawluski, J.L., Lambert, K.G., Kinsley, C.H., 2016. Neuroplasticity in the maternal hippocampus: Relation to cognition and effects of repeated stress. Horm. Behav. 77, 86–97. https://doi.org/10.1016/j.yhbeh.2015.06.004

Pawluski, J.L., Li, M., Lonstein, J.S., 2019. Serotonin and motherhood: From molecules to mood. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2019.03.001

Pereira, V.S., Hiroaki-Sato, V.A., 2018. A brief history of antidepressant drug development: From tricyclics to beyond ketamine. Acta Neuropsychiatr. 30, 307–322. https://doi.org/10.1017/neu.2017.39

Perfalk, E., Cunha-Bang, S. da, Holst, K.K., Keller, S., Svarer, C., Knudsen, G.M., Frokjaer, V.G., 2017. Testosterone levels in healthy men correlate negatively with serotonin 4 receptor binding. Psychoneuroendocrinology 81, 22–28. https://doi.org/10.1016/j.psyneuen.2017.03.018

Pletzer, B., Harris, T., Hidalgo-Lopez, E., 2018. Subcortical structural changes along the menstrual cycle: beyond the hippocampus. Sci. Rep. 8, 16042. https://doi.org/10.1038/s41598-018-34247-4

Pletzer, B., Harris, T.A., Hidalgo-Lopez, E., 2019. Previous contraceptive treatment relates to grey matter volumes in the hippocampus and basal ganglia. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-47446-4

Pletzer, B., Kronbichler, M., Aichhorn, M., Bergmann, J., Ladurner, G., Kerschbaum, H.H., 2010. Menstrual cycle and hormonal contraceptive use modulate human brain structure. Brain Res. 1348, 55–62. https://doi.org/10.1016/j.brainres.2010.06.019

Potter, W.Z., Scheinin, M., Golden, R.N., Rudorfer, M. V., Cowdry, R.W., Calil, H.M., Ross, R.J., Linnoila, M., 1985. Selective Antidepressants and Cerebrospinal Fluid: Lack of Specificity on Norepinephrine and Serotonin Metabolites. Arch. Gen. Psychiatry 42, 1171–1177. https://doi.org/10.1001/ARCHPSYC.1985.01790350045009

Praschak-Rieder, N., Kennedy, J., Wilson, A.A., Hussey, D., Boovariwala, A., Willeit, M., Ginovart, N., Tharmalingam, S., Masellis, M., Houle, S., Meyer, J.H., 2007. Novel 5-HTTLPR Allele Associates with Higher Serotonin Transporter Binding in Putamen: A [11C] DASB Positron Emission Tomography Study. Biol. Psychiatry 62, 327–331. https://doi.org/10.1016/j.biopsych.2006.09.022

Protopopescu, X., Butler, T., Pan, H., Root, J., Altemus, M., Polanecsky, M., McEwen, B., Silbersweig, D., Stern, E., 2008. Hippocampal structural changes across the menstrual cycle. Hippocampus 18, 985–988. https://doi.org/10.1002/hipo.20468

Puonti, O., Iglesias, J.E., Van Leemput, K., 2016. Fast and sequence-adaptive whole-brain segmentation using parametric Bayesian modeling. Neuroimage 143, 235–249. https://doi.org/10.1016/j.neuroimage.2016.09.011

Putnam, K.T., Wilcox, M., Robertson-Blackmore, E., Sharkey, K., Bergink, V., Munk-Olsen, T., Deligiannidis, K.M., Payne, J., Altemus, M., Newport, J., Apter, G., Devouche, E., Viktorin, A., Magnusson, P., Penninx, B., Buist, A., Bilszta, J., O'Hara, M., Stuart, S., Brock, R., Roza, S., Tiemeier, H., Guille, C., Epperson, C.N., Kim, D., Schmidt, P., Martinez, P., Di Florio, A., Wisner, K.L., Stowe, Z., Jones, I., Sullivan, P.F., Rubinow, D., Wildenhaus, K., Meltzer-Brody, S., 2017. Clinical phenotypes of perinatal depression and time of symptom onset: analysis of data from an international consortium. The Lancet Psychiatry 4, 477–485. https://doi.org/10.1016/S2215-0366(17)30136-0

Qiu, W., Hodges, T.E., Clark, E.L., Blankers, S.A., Galea, L.A.M., 2020. Perinatal depression:

Heterogeneity of disease and in animal models. Front. Neuroendocrinol. 59, 100854. https://doi.org/10.1016/j.yfrne.2020.100854

Reck, C., Stehle, E., Reinig, K., Mundt, C., 2009. Maternity blues as a predictor of DSM-IV depression and anxiety disorders in the first three months postpartum. J. Affect. Disord. 113, 77–87. https://doi.org/10.1016/j.jad.2008.05.003

Rehbein, E., Hornung, J., Sundström Poromaa, I., Derntl, B., 2021. Shaping of the Female Human Brain by Sex Hormones: A Review. Neuroendocrinology 111, 183–206. https://doi.org/10.1159/000507083

Richter, D., Krämer, M.D., Tang, N.K.Y., Montgomery-Downs, H.E., Lemola, S., 2019. Long-term effects of pregnancy and childbirth on sleep satisfaction and duration of first-time and experienced mothers and fathers. SLEEPJ 42. https://doi.org/10.1093/sleep/zsz015

Robertson, E., Grace, S., Wallington, T., Stewart, D.E., 2004. Antenatal risk factors for postpartum depression: A synthesis of recent literature. Gen. Hosp. Psychiatry 26, 289–295. https://doi.org/10.1016/j.genhosppsych.2004.02.006

Roux, C.M., Leger, M., Freret, T., 2021. Memory disorders related to hippocampal function: The interest of 5-ht4 rs targeting. Int. J. Mol. Sci. 22, 12082. https://doi.org/10.3390/ijms222112082

Sacher, J., Rekkas, P.V., Wilson, A.A., Houle, S., Romano, L., Hamidi, J., Rusjan, P., Fan, I., Stewart, D.E., Meyer, J.H., 2015. Relationship of monoamine oxidase-A distribution volume to postpartum depression and postpartum crying. Neuropsychopharmacology 40, 429–435. https://doi.org/10.1038/npp.2014.190

Sacher, J., Wilson, A.A., Houle, S., Rusjan, P., Hassan, S., Bloomfield, P.M., Stewart, D.E., Meyer, J.H., 2010. Elevated brain monoamine oxidase A binding in the early postpartum period. Arch. Gen. Psychiatry 67, 468–474. https://doi.org/10.1001/archgenpsychiatry.2010.32

Salari, N., Darvishi, N., Khaledi-Paveh, B., Vaisi-Raygani, A., Jalali, R., Daneshkhah, A., Bartina, Y., Mohammadi, M., 2021. A systematic review and meta-analysis of prevalence of insomnia in the third trimester of pregnancy. BMC Pregnancy Childbirth 21, 1–8. https://doi.org/10.1186/s12884-021-03755-z

Sanjuán, J., Martin-Santos, R., Garcia-Esteve, L., Carot, J.M., Guillamat, R., Gutierrez-Zotes, A., Gornemann, I., Canellas, F., Baca-Garcia, E., Jover, M., Navines, R., Valles, V., Vilella, E., De

Diego, Y., Castro, J.A., Ivorra, J.L., Gelabert, E., Guitart, M., Labad, A., Mayoral, F., Roca, M., Gratacos, M., Costas, J., Van Os, J., De Frutos, R., 2008. Mood changes after delivery: Role of the serotonin transporter gene. Br. J. Psychiatry 193, 383–388. https://doi.org/10.1192/bjp.bp.107.045427

Schmaal, L., Veltman, D.J., Van Erp, T.G.M., Smann, P.G., Frodl, T., Jahanshad, N., Loehrer, E., Tiemeier, H., Hofman, A., Niessen, W.J., Vernooij, M.W., Ikram, M.A., Wittfeld, K., Grabe, H.J., Block, A., Hegenscheid, K., Völzke, H., Hoehn, D., Czisch, M., Lagopoulos, J., Hatton, S.N., Hickie, I.B., Goya-Maldonado, R., Krmer, B., Gruber, O., Couvy-Duchesne, B., Rentera, M.E., Strike, L.T., Mills, N.T., De Zubicaray, G.I., McMahon, K.L., Medland, S.E., Martin, N.G., Gillespie, N.A., Wright, M.J., Hall, G.B., MacQueen, G.M., Frey, E.M., Carballedo, A., Van Velzen, L.S., Van Tol, M.J., Van der Wee, N.J., Veer, I.M., Walter, H., Schnell, K., Schramm, E., Normann, C., Schoepf, D., Konrad, C., Zurowski, B., Nickson, T., McIntosh, A.M., Papmeyer, M., Whalley, H.C., Sussmann, J.E., Godlewska, B.R., Cowen, P.J., Fischer, F.H., Rose, M., Penninx, B.W.J.H., Thompson, P.M., Hibar, D.P., 2016. Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. Mol. Psychiatry 21, 806–812. https://doi.org/10.1038/mp.2015.69

Schock, H., Zeleniuch-Jacquotte, A., Lundin, E., Grankvist, K., Lakso, H.Å., Idahl, A., Lehtinen, M., Surcel, H.M., Fortner, R.T., 2016. Hormone concentrations throughout uncomplicated pregnancies: A longitudinal study. BMC Pregnancy Childbirth 16, 1–11. https://doi.org/10.1186/s12884-016-0937-5

Sederevičius, D., Vidal-Piñeiro, D., Sørensen, Ø., van Leemput, K., Iglesias, J.E., Dalca, A. V, Greve, D.N., Fischl, B., Bjørnerud, A., Walhovd, K.B., Fjell, A.M., 2021. Reliability and sensitivity of two whole-brain segmentation approaches included in FreeSurfer – ASEG and SAMSEG. Neuroimage 237, 118113. https://doi.org/10.1016/j.neuroimage.2021.118113

Sharp, T., Barnes, N.M., 2020. Central 5-HT receptors and their function; present and future. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2020.108155

Sheehan, D. V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. 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, 22–33.

Sheline, Y.I., Bardgett, M.E., Csernansky, J.G., 1997. Correlated Reductions in Cerebrospinal Fluid 5-HIAA and MHPG Concentrations After Treatment With Selective Serotonin Reuptake Inhibitors. J. Clin. Psychopharmacol. 17, 11–14. https://doi.org/10.1097/00004714-199702000-00003

Sheppard, P.A.S., Choleris, E., Galea, L.A.M., 2019. Structural plasticity of the hippocampus in response to estrogens in female rodents. Mol. Brain. https://doi.org/10.1186/s13041-019-0442-7

Skovlund, C.W., Mørch, L.S., Kessing, L.V., Lidegaard, O., 2016. Association of hormonal contraception with depression. JAMA Psychiatry 73, 1154–1162. https://doi.org/10.1001/jamapsychiatry.2016.2387

Smith-Nielsen, J., Matthey, S., Lange, T., Væver, M.S., 2018. Validation of the Edinburgh Postnatal Depression Scale against both DSM-5 and ICD-10 diagnostic criteria for depression. BMC Psychiatry 18, 393. https://doi.org/10.1186/s12888-018-1965-7

Soares, C.N., Zitek, B., 2008. Reproductive hormone sensitivity and risk for depression across the female life cycle: A continuum of vulnerability?, in: Journal of Psychiatry and Neuroscience. Canadian Medical Association, pp. 331–343.

Sohrabji, F., 2005. Estrogen: A neuroprotective or proinflammatory hormone? Emerging evidence from reproductive aging models. Ann. N. Y. Acad. Sci. 1052, 75–90. https://doi.org/10.1196/annals.1347.006

Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R., Jacobs, G.A., 1983. Manual for the State-Trait Anxietry, Inventory. Palo Alto, CA Consult. Psychol. Press.

Spielman, F.J., Mueller, R.A., Corke, B.C., 1985. Cerebrospinal fluid concentration of 5-hydroxyindoleacetic acid in pregnancy. Anesthesiology 62, 193–195. https://doi.org/10.1097/00000542-198502000-00022

Stein, A., Pearson, R.M., Goodman, S.H., Rapa, E., Rahman, A., McCallum, M., Howard, L.M., Pariante, C.M., 2014. Effects of perinatal mental disorders on the fetus and child. Lancet. https://doi.org/10.1016/S0140-6736(14)61277-0

Stenbæk, D.S., Fisher, P.M., Ozenne, B., Andersen, E., Hjordt, L. V., McMahon, B., Hasselbalch, S.G., Frokjaer, V.G., Knudsen, G.M., 2017. Brain serotonin 4 receptor binding is inversely associated with verbal memory recall. Brain Behav. 7. https://doi.org/10.1002/brb3.674

Suda, S., Segi-Nishida, E., Newton, S.S., Duman, R.S., 2008. A Postpartum Model in Rat: Behavioral and Gene Expression Changes Induced by Ovarian Steroid Deprivation. Biol. Psychiatry 64, 311–319. https://doi.org/10.1016/j.biopsych.2008.03.029

Sumner, B.E.H., Grant, K.E., Rosie, R., Hegele-Hartung, C., Fritzemeier, K.H., Fink, G., 2007. Raloxifene blocks estradiol induction of the serotonin transporter and 5-hydroxytryptamine2A receptor in female rat brain. Neurosci. Lett. 417, 95–99. https://doi.org/10.1016/j.neulet.2007.02.039

Svarer, C., Madsen, K., Hasselbalch, S.G., Pinborg, L.H., Haugbøl, S., Frøkjær, V.G., Holm, S., Paulson, O.B., Knudsen, G.M., 2005. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 24, 969–979. https://doi.org/10.1016/j.neuroimage.2004.10.017

T. Abou-Saleh, M., Ghubash, R., Karim, L., Krymski, M., Bhai, I., 1998. Hormonal aspects of postpartum depression. Psychoneuroendocrinology 23, 465–475. https://doi.org/10.1016/S0306-4530(98)00022-5

Tavares Pinheiro, R., Monteiro Da Cunha Coelho, F., Azevedo Da Silva, R., Amaral Tavares Pinheiro, K., Pierre Oses, J., De Ávila Quevedo, L., Dias De Mattos Souza, L., Jansen, K., Maria Zimmermann Peruzatto, J., Gus Manfro, G., Giovenardi, M., Almeida, S., Lucion, A.B., 2013. Association of a serotonin transporter gene polymorphism (5-HTTLPR) and stressful life events with postpartum depressive symptoms: A population-based study. J. Psychosom. Obstet. Gynecol. 34, 29–33. https://doi.org/10.3109/0167482X.2012.759555

Tendais, I., Costa, R., Conde, A., Figueiredo, B., 2014. Screening for depression and anxiety disorders from pregnancy to postpartum with the EPDS and STAI. Span. J. Psychol. 17, E7. https://doi.org/10.1017/sjp.2014.7

Thomas, E.J., Jenkins, J., Lenton, E.A., Cooke, I.D., 1986. Endocrine effects of goserelin, a new depot luteinising hormone releasing hormone agonist. Br. Med. J. (Clin. Res. Ed). 293, 1407–1408. https://doi.org/10.1136/bmj.293.6559.1407-a

Toriola, A.T., Vääräsmäki, M., Lehtinen, M., Zeleniuch-Jacquotte, A., Lundin, E., Rodgers, K.G., Lakso, H.A., Chen, T., Schock, H., Hallmans, G., Pukkala, E., Toniolo, P., Grankvist, K., Surcel, H.M., Lukanova, A., 2011. Determinants of maternal sex steroids during the first half of pregnancy. Obstet. Gynecol. 118, 1029–1036.

https://doi.org/10.1097/AOG.0b013e3182342b7f

Van Niel, M.S., Payne, J.L., 2020. Perinatal depression: A review. Cleve. Clin. J. Med. 87, 273–277. https://doi.org/10.3949/ccjm.87a.19054

Videbech, P., Ravnkilde, B., 2004. Hippocampal volume and depression: A meta-analysis of MRI studies. Am. J. Psychiatry. https://doi.org/10.1176/appi.ajp.161.11.1957

Weikop, P., Kehr, J., Scheel-Krüger, J., 2007. Reciprocal effects of combined administration of serotonin, noradrenaline and dopamine reuptake inhibitors on serotonin and dopamine levels in the rat prefrontal cortex: the role of 5-HT 1A receptors. J. Psychopharmacol. 21, 795–804. https://doi.org/10.1177/0269881107077347

Welch, B.L., 1947. THE GENERALIZATION OF 'STUDENT'S' PROBLEM WHEN SEVERAL DIFFERENT POPULATION VARLANCES ARE INVOLVED. Biometrika 34, 28–35. https://doi.org/10.1093/biomet/34.1-2.28

Williams, R.B., Marchuk, D.A., Gadde, K.M., Barefoot, J.C., Grichnik, K., Helms, M.J., Kuhn, C.M., Lewis, J.G., Schanberg, S.M., Stafford-Smith, M., Suarez, E.C., Clary, G.L., Svenson, I.K., Siegler, I.C., 2003. Serotonin-related gene polymorphisms and central nervous system serotonin function. Neuropsychopharmacology 28, 533–541. https://doi.org/10.1038/sj.npp.1300054

Willner, P., Scheel-Krüger, J., Belzung, C., 2013. The neurobiology of depression and antidepressant action. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2012.12.007

wilson, M., Morganti, A.A., Zervoudakis, I., Letcher, R.L., Romney, B.M., Von Oeyon, P., Papera, S., Sealey, J.E., Laragh, J.H., 1980. Blood pressure, the renin-aldosterone system and sex steroids throughout normal pregnancy. Am. J. Med. 68, 97–104. https://doi.org/10.1016/0002-9343(80)90178-3

Wisner, K.L., Perel, J.M., Peindl, K.S., Hanusa, B.H., 2004. Timing of depression recurrence in the first year after birth. J. Affect. Disord. 78, 249–252. https://doi.org/10.1016/S0165-0327(02)00305-1

Woods, R.P., Cherry, S.R., Mazziotta, J.C., 1992. Rapid automated algorithm for aligning and reslicing pet images. J. Comput. Assist. Tomogr. 16, 620–633. https://doi.org/10.1097/00004728-199207000-00024

Woolley, C., McEwen, B., 1992. Estradiol mediates fluctuation in hippocampal synapse density

during the estrous cycle in the adult rat. J. Neurosci. 12, 2549. https://doi.org/10.1523/JNEUROSCI.12-07-02549.1992

World Health Organisation, 1992. The ICD-10 Classification of Mental and Behavioural Disorders. World Health Organization.

Y, S., ME, B., JG, C., 1997. Correlated reductions in cerebrospinal fluid 5-HIAA and MHPG concentrations after treatment with selective serotonin reuptake inhibitors. J. Clin. Psychopharmacol. 17, 11–14. https://doi.org/10.1097/00004714-199702000-00003

Yankova, M., Hart, S.A., Woolley, C.S., 2001. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: A serial electron-microscopic study. Proc. Natl. Acad. Sci. U. S. A. 98, 3525–3530. https://doi.org/10.1073/pnas.051624598

Zhang, Z., Hong, J., Zhang, S., Zhang, T., Sha, S., Yang, R., Qian, Y., Chen, L., 2016. Postpartum estrogen withdrawal impairs hippocampal neurogenesis and causes depression- and anxiety-like behaviors in mice. Psychoneuroendocrinology 66, 138–149. https://doi.org/10.1016/j.psyneuen.2016.01.013

Zimmerman, M., Martinez, J.H., Young, D., Chelminski, I., Dalrymple, K., 2013. Severity classification on the Hamilton depression rating scale. J. Affect. Disord. 150, 384–388. https://doi.org/10.1016/j.jad.2013.04.028

Manuscripts

Title: Hippocampal volume brain changes in a pharmacological sex-hormone manipulation model for depression in women

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Abstract

Hormone transition phases may trigger depression in some women, yet the underlying mechanisms remain elusive. In a pharmacological sex-hormone manipulation model, we previously reported that estradiol reductions, induced with a gonadotropin-releasing hormone agonist (GnRHa), provoked subclinical depressive symptoms in healthy women, especially in women who increased in brain serotonin transporter (SERT) binding. Within this model, we here evaluated if GnRHa, compared to placebo, reduced hippocampal volume, in a manner that depended on the magnitude of the estradiol decrease and SERT availability, and if this decrease translated to the emergence of subclinical depressive symptoms.

Sixty-three healthy, naturally cycling women were included in a randomized, double-blind, placebo-controlled GnRHa-intervention study. We quantified the change from baseline to follow-up (n = 60) in serum estradiol, brain SERT levels (positron emission tomography with [^{11}C]DASB), depressive symptoms (Hamilton depression rating scale), and hippocampal volume (magnetic resonance imaging; volume quantified in Freesurfer 7.1). Group differences in hippocampal volume changes was evaluated in a t-test. We evaluated potential associations between changes in estradiol, hippocampal volume and depressive symptoms and potential SERT-by-estradiol interaction effects in linear regression models.

GnRHa did not reduce hippocampal volume relative to placebo. There was no interaction effect between GnRHa-induced estradiol changes and brain SERT on hippocampal volume. Hippocampal volume decreased in response to GnRHa-induced reductions in estradiol ($p = 0.04$; Cohen's $f^2 = 0.18$), controlled for baseline SERT binding, but was not associated with depressive symptoms .

If replicated, our data highlight a possible association between estradiol fluctuations and hippocampal plasticity, adjusted for serotonergic contributions.

Keywords *Hippocampus; Estradiol; Gonadotropin-releasing-hormone agonist; Hormones; Serotonin transporter; Women; MRI; PET; Brain; Depression*

Introduction

Major depressive disorder is one of the highest-ranking causes of disability worldwide(Abbafati et al., 2020). Strikingly, it affects twice as many women than men after puberty (Kuehner, 2017). In particular, women experience a heightened risk during hormonal transition phases, i.e., pregnancy and birth or perimenopause (Freeman et al., 2014; Lokuge et al., 2011; Munk-Olsen et al., 2006). The exact risk and disease mechanisms for depressive episodes related to hormonal transitions remain elusive, however brain plasticity may play a role as shown in rodent models (Eid et al., 2019; Medina and Workman, 2020; Pawluski et al., 2016; Sheppard et al., 2019; Yagi and Galea, 2019). Such neuroplastic effects of sex-hormone fluctuations are not surprising, as sex-steroid receptors are highly expressed in the hippocampus, where especially estrogen affects synaptic remodeling and neurogenesis (Barth et al., 2015; Gould et al., 2000; Yankova et al., 2001). Correspondingly, human studies support that the dramatic changes in sex-hormone milieu during pregnancy and birth are associated with a reorganization of the brain , such as decreased hippocampal volume in the early postpartum period relative to preconception for first time mothers, which is normalized within two years postpartum (Hoekzema et al., 2017; Martínez-García et al., 2021; Oatridge et al., 2002). Similar brain maturation trajectories are observed during female pubertal transition, which is characterized by a long-term increase in sex-hormones (Cameron, 2004; Carmona et al., 2019; Sisk and Zehr, 2005). On the other hand, dynamic fluctuations of estradiol over the menstrual cycle are associated with rapid synchronous hippocampal volume changes, i.e., they both peak just before ovulation (Barth et al., 2016; Lisofsky et al., 2015; Pletzer et al., 2018; Protopopescu et al., 2008; Woolley and McEwen, 1992). Further, in perimenopause, higher doses of exogenous estrogens are linked to increased hippocampal volume and protection of cognitive functioning (Albert et al., 2017; Lord et al., 2008; Sohrabji, 2005). Several papers demonstrate that these neuroprotective effects of estrogens occur in interaction with serotonergic neurotransmission (Amin et al., 2006; Barth et al., 2015; Bethea et al., 2009; Frokjaer et al., 2010). Intriguingly, numerous studies report a decreased hippocampal volume in patients with depression, compared to healthy individuals (Enneking et al., 2019; McKinnon et al., 2009; Schmaal et al., 2016; Videbech and Ravnkilde, 2004; Willner et al., 2013). Hence, the emergence of depressive symptoms in response to sex steroid transitions may be

associated with decreasing hippocampal volume possibly in concert with altered serotonergic signaling.

We have developed a pharmacological sex-hormone manipulation model conceptualized to study sex-steroid related risk mechanisms for depression (Frokjaer, 2020). In this model, we used a gonadotrophin-releasing hormone agonist (GnRHa) intervention to induce a biphasic estradiol fluctuation in healthy women of reproductive age. Between baseline and follow-up, participant who received GnRHa went through a brief sex-hormone axis stimulation phase, followed by complete suppression. This resulted in a net decrease in estradiol from baseline to follow-up (mean change 0.12 nmol/L). GnRHa, relative to placebo, provoked subclinical depressive symptoms in 12% of healthy volunteers. The emergence of depressive symptoms, from before intervention to the suppressed state, within the GnRHa group was associated with the magnitude of the estradiol decline, i.e., women who experienced a large decrease in estradiol developed more depressive symptoms, than those who experienced a small decrease. The depressogenic estradiol effect was coupled to an increase in brain levels of the serotonin transporter (SERT), which is the main regulator of synaptic serotonin (Frokjaer et al., 2015). This phenomenon was associated with estrogen sensitivity markers, that identifies hormone sensitive individuals, at the level of DNA methylation and gene expression, known from clinical cohorts of postpartum depression as well (Mehta et al., 2018, 2014). Further, we observed changes in both task-based and resting state fMRI, including a mood and estradiol dependent functional decoupling of hippocampus and cingulate cortex at rest (Fisher et al., 2017; Henningsson et al., 2015; Macoveanu et al., 2016). The potential impact of GnRHa on brain structure, i.e., hippocampus volumes, in this dataset has not yet been determined. We here evaluated if a placebo-controlled GnRHa-intervention in 60 healthy women, reduced hippocampal volume and if such hippocampal volume changes were associated with the GnRHa-induced decrease in estradiol ,. Further, we evaluated if this phenomenon was associated with GnRHa-induced depressive symptoms. We hypothesized that hippocampal volume would decrease in response to GnRHa relative to placebo, in a manner that depended on the magnitude of the GnRHa-induced decrease in estradiol, and that this hippocampal volume decrease might be positively associated with the emergence of depressive symptoms in the GnRHa-group. To test the hypothesis that such associations were

dependent on serotonin brain architecture, we also evaluated if SERT binding in the GnRHa group interacted with the estradiol changes on hippocampal volume.

Methods:

Participants and design

The study population of naturally cycling healthy women is described in detail in Frokjaer et al. (2015; clinicaltrials.gov ID: NCT02661789). Briefly, 63 healthy premenopausal women were included in a double-blind, placebo-controlled block randomized intervention study registered and approved by the local ethics committee (protocol-ID: H-2-2010-108). All participants gave written informed consent. Inclusion criteria were: regular menstrual cycle with a length of 23-35 days, no history of neurological or psychiatric disorders, no history of premenstrual dysphoric disorder, no alcohol abuse or use of illegal drugs and a normal physical, neurological and gynecological examination. Participants were block randomized to either placebo or GnRHa and with respect to serotonin transporter-linked polymorphic region (5-HTTLPR) genotype, for purposes related to previously published data (Frokjaer et al., 2015). For an overview of the study design, see Figure 1.

Baseline assessments were completed on menstrual cycle day 5.2±2.1 (midfollicular phase) and included a 17-item Hamilton Depression Rating Scale (HAMILTON, 1967) interview with a trained clinician, structural magnetic resonance imaging (MRI) for hippocampal volume assessment, [^{11}C]DASB positron emission tomography (PET) scans for brain SERT quantification, and blood samples for estradiol concentration measurements. 60 women (placebo n=29, GnRHa n=31) completed follow-up interviews and blood samples 16.3±2.7 days after intervention, and MRI scans 16±2.8 days after intervention. This corresponded to the follicular phase in the placebo group. For most women, approximately 30 days (one menstrual cycle) elapsed between baseline and follow-up, although for a few women, the intervention was delayed and had to be administered in another cycle. The majority of MR scans were collected on the same day as blood samples, PET scans and interviews (mean deviation from MR date: 0.07±2.5 days; range ± 9 days). Three women did not complete follow-up MRI: (1) One was excluded due to anovulation prior to

intervention, (2) one conceived a child, and (3) one participant was unable to attend the MR scan at follow-up.

Intervention and hormone analyses

A gynecologist, not otherwise involved in project, administered either placebo (saline) or a GnRHa implant (ZOLADEX, a biodegradable copolymer impregnated with 3.6 mg of goserelin; AstraZeneca, London, United Kingdom) to the participants on menstrual cycle day 22.75 ± 2.77 (post ovulation state was ultrasound verified). Serum samples for hormone analyses were collected at baseline and follow-up and analyzed within 24 hours of collection. We used an electrochemiluminescence immunoassay to assess estradiol concentrations on Modular Analytics Serum Work Area equipment (Roche, Mannheim, Germany; detection range 0.04 and 78.9 nmol/L). For the purpose of statistical analysis, estradiol concentrations below the detection level were imputed to 0.04 nmol/L.

Neuroimaging

At baseline and follow-up, we acquired T1-weighted sagittal magnetization prepared rapid gradient echo (MPRAGE) structural MR images on a 3T Verio scanner with a 32-channel head array coil (Siemens, Erlangen, Germany). Imaging parameters were: echo time (TE) = 2.32; repetition time (TR) = 1900; inversion time (TI) = 900 ms; flip angle= 9°; in-plane matrix: 256x256; in-plane resolution: 0.9x0.9mm; slice thickness = 0.9 mm; 224 slices, no gap.

Automatic subcortical segmentation of MR images was performed in Freesurfer version 7.1 (http://surfer.nmr.mgh.harvard.edu/; Fischl et al., 2002). All images were inspected for segmentation errors by two of the authors and, if relevant, such errors were manually edited before re-running the pipeline. Left and right hippocampal volumes were extracted for statistical analysis.

Methods and primary results for [^{11}C]DASB-PET are reported in Frokjaer et al. (2015). In the current analysis, we evaluated if including in vivo levels of SERT in neocortex, measured as [^{11}C]DASB-PET binding potentials, significantly contributed to any of our models (see statistical analysis section).

Statistical analysis

Prior to analyses, we evaluated covariates for non-normality and found that estradiol concentrations were not normally distributed. Hence, normally distributed log-transformed (base = 2) estradiol concentrations were used for statistical analyses.

To determine if there were any baseline effects irrespective of GnRHa sex-hormone manipulation, in order to specify our model, we used a linear regression to evaluate if hippocampal volume was associated with three potential variables: follicular phase estradiol concentrations, SERT binding potentials, and age. Inclusion of age as a covariate was motivated by the known age-related hippocampal decline, also in young individuals (Fraser et al., 2015; Sederevičius et al., 2021). All three contributed to baseline hippocampal volume and were considered for the main analysis.

In line with the general analysis structure for the study (Frokjaer et al., 2015) *changes* from baseline to rescan in hippocampal volume, in estradiol concentration (within GnRHa group only) and in Hamilton score were used in all main analyses. First, we evaluated if there was an effect of GnRHa compared to placebo in hippocampal volume change with a Welch two-sample t-test (Welch, 1947). Second, for the GnRHa group we used multiple linear regression models to determine if a) GnRHa-induced change in estradiol, alone or in interaction with change in SERT binding or SERT at baseline, was associated with hippocampal volume, and b) if hippocampal volume changes, alone or in interaction with estradiol changes, were associated with the emergence of depressive symptoms, within the GnRHa group. For robustness analysis purposes, all of these models were reviewed for potential contributions from age. We supplemented this with a structural equation modeling in the R (http://cran.r-project.org/) package *lava* (Holst and Budtz-Jørgensen, 2012), to evaluate if hippocampal volume changes might mediate the association between estradiol changes and the emergence of depressive symptoms.

In the GnRHa study, perfect timing with regard to baseline cycle or intervention could not be obtained for all assessments. Time delays of more than five days between MR scans and either blood samples, psychometrics or PET scans were considered potentially problematic for association analyses. Thus, data from individuals, where these measures differed more than five days from each other, were ignored in both groups at baseline (n = 3, all in GnRHa group) and at follow-up in the placebo group (n = 1). They were not ignored for the GnRHa group at follow-up,

as participants were considered fully downregulated in their estradiol concentrations for the entire period (Thomas et al., 1986). Thus, four affected participants were ignored in change analyses (GnRHa: n = 3, placebo: n = 1). This cut-off was chosen as a conservative approach to the closeness of observations. For analyses including only age or group as covariates, all available data points were included.

Similarly, we used a linear mixed effects model to evaluate if there was a potential lateralization of the group difference, the association between hippocampal volume and estradiol, and the association between hippocampal volume and depressive symptoms, but found no evidence for a lateralization. Log-likelihood ratio tests supported that the inclusion of left and right hippocampus separately did not improve model fit for the association with depressive symptoms, compared to mean hippocampal volume pooled from both brain hemispheres. Thus, we use a mean of left and right hippocampus for all analyses.

The reported p-values were not adjusted for multiple comparisons and p-values < 0.05 were considered statistically significant. We chose this approach as the study was driven by few *a priori* hypotheses, supplemented with robustness analysis, in a dataset that can be considered preliminary. See "Methodological considerations" in the discussion. Effect size is reported as either Cohen's *d* (t-tests) or Cohen's f^2 (linear regression models).

Results:

Demographics and baseline effects

Data on demographics, hormone levels and mean hippocampal volume for the two groups can be found in Table 1. At inclusion, the median age of participants was 22.4 years old (range: [18.4; 37.2]). Participants in the GnRHa-group were slightly younger and had slightly larger mean hippocampal volumes at baseline (mean difference: 118 mm3, p = 0.22, Table 1).

In a multiple linear regression model that included age, SERT binding at baseline and estradiol at baseline, we observed significant associations between hippocampal volume and both age, SERT binding, and estradiol concentrations ($\beta_{estradiol}$: 281.78, 95% CI: [62.173; 501.394], p = 0.01, Cohen's f^2 = 0.13; β_{age}: -43.59, 95% CI: [-63.937; -23.244], p < 0.001, Cohen's f^2 = 0.35 ; β_{SERT}: -2298.81; 95% CI: [-3723.469; -874.156], p = 0.002, Cohen's f^2 = 0.2, Figure 2). As expected, this association was negative for age. SERT binding was also negatively associated with hippocampal volume at baseline, while estradiol was positively associated with hippocampal volume. For age and SERT binding, but not estradiol, these effects were also present in models that were not adjusted for other variables, but at a less significant level (β_{age} = -25.39; 95% CI: [-44.398; -6.383], p = 0.01, Cohen's f^2 = 0.13 ; β_{SERT} = - 1786.4, p = 0.03, Cohen's f^2 = 0.1 ; $\beta_{estradiol}$ = 69.88, 95% CI: [-161.544; 293.3], p = 0.57, Cohen's f^2 = 0.01).

Effect of intervention on hippocampal volume

There was a statistically non-significant mean decrease in hippocampal volume the GnRHa group (mean change = -26.77 mm3), compared to the placebo group, i.e., in the expected direction (mean difference in change = 37.9 mm3, 95% CI: [-13.155; 88.956], p = 0.14, Cohen's *d* = 0.38, Figure 3).

Hippocampal volume changes in the GnRHa group and associations with the emergence of depressive symptoms and change in estradiol

Association between change in hippocampal volume and change in estradiol in the GnRHa group and interaction with serotonin brain binding

Change in estradiol had no interaction effects with change in SERT binding ($\beta_{\Delta SERT}$ = 1420.30, 95% CI: [-1333.41; 4174.01], p = 0.3, Cohen's f^2 = 0.05) or SERT binding at baseline (-234.6, 95% CI: [-1859.65; 1390.43], p = 0.77, Cohen's f^2 = 0.01) on hippocampal volume. However, in a model that included both baseline levels of SERT and change in estradiol, there was a significant association between hippocampal volume and change in estradiol($\beta_{\Delta estradiol}$ = 56.93, 95% CI: [1.98; 111.89], p = 0.04, Cohen's f^2 = 0.18; $\beta_{SERT\_baseline}$ = 698.60, 95% CI: [22.97; 1374.24], p = 0.04, Cohen's f^2 = 0.18, Figure 5). No such effects were found when change in SERT was included ($\beta_{estradiol}$ = 48.5, 95% CI: [-10.48; 107.48], p = 0.1, Cohen's f^2 = 0.12 ; $\beta_{\Delta SERT}$ = 148.53, 95% CI: [-692.2; 989.27], p = 0.72, Cohen's f^2 = 0.005). Age was not associated with change in hippocampal volume in any of these models (p-values > 0.5) and adjusting for age had no substantial effect on the association between change in estradiol and change in hippocampal volume.

Association between changes in hippocampal volume and the emergence of subclinical depressive symptoms in the GnRHa group

We previously published an increase in depressive symptoms, measured as Hamilton score, in response to GnRHa compared to placebo (Frokjaer et al., 2015). Here, we observed no significant association between change in hippocampal volume and the emergence of depressive symptoms within the GnRHa group, adjusted for estradiol change ($\beta_{\Delta Hippocampus}$ = 0.01, 95% CI: [-0.006; 0.025], p = 0.21, Cohen's f^2 = 0.07, Figure 4). Further, we did not observe a significant interaction effect between change in estradiol (dichotomized with a median split) and hippocampal volume on depressive symptoms in the GnRHa group ($\beta_{estradiol\ large\ decrease\text{-}by\text{-}\Delta hippocampal\ volume}$ = 0.009, 95% CI: [-0.02; 0.04], p = 0.53, Cohen's f^2 = 0.02). Inclusion of change in SERT or SERT at baseline did not substantially change these results (all p-values > 0.2).

In structural equation models, we found no evidence that hippocampal volume change mediated the association between estradiol changes and the emergence of depressive symptoms (estimate. 0.55, mediation proportion: -0.25 with 95% CI [-0.76; 0.26], p = 0.24).

Discussion:

We here present data on hippocampal volume changes in response to a placebo-controlled GnRHa sex-hormone manipulation in healthy women. We were not able to confirm a main effect of decreased hippocampal volume in response to the sex-hormone manipulation. Nor did we observe any evidence that hippocampal volume mediated the previously published association between depressive symptoms and change in estradiol, within the GnRHa group. However, within the GnRHa group, hippocampal volume decrease was associated with the magnitude of the estradiol decline , when taking SERT binding at baseline into account. Contrary to our hypothesis, this did not translate to the emergence of subclinical depressive symptoms in the GnRHa group. At baseline, significant associations between age, estradiol, SERT binding and hippocampal volumes were demonstrated.

Sex-hormone manipulation and changes in hippocampal volume

Although in the proposed direction, the group difference in hippocampal volume was not statistically significant. However, the GnRHa group decreased approximately 30 mm3 more than the placebo group, which is of similar magnitude to changes seen across the menstrual cycle or the absolute difference between depressed and healthy individuals (Lisofsky et al., 2015; Schmaal et al., 2016). Thus, we may simply not have the power to detect such an effect. Notably, many factors regulate hippocampal volume, and estradiol changes of the magnitude experienced in the GnRHa group may play only a minor role. . However, association analyses within the GnRHa group, showed that GnRHa-induced change in estradiol, controlled for SERT binding at baseline, and change in hippocampal volume were positively associated. That is, the greater the magnitude of the decrease in estradiol, the greater the decrease in hippocampal volume. This suggests a dose-response relation between changes in estradiol concentration and hippocampal volume, when taking baseline SERT contributions in to account. Even the limited variability in follicular phase estradiol concentrations, measured at baseline, translated to absolute hippocampal volume variations, controlled for SERT binding and age, such that women with higher estradiol concentrations had larger hippocampal volumes ($p = 0.01$). This result should be interpreted with care, as it originated from model qualification analyses and not an a priori hypothesis. The results are however in line with studies of naturally cycling women, where hippocampal volume is

reduced during phases with low estradiol levels and vice versa in periods with increasing estradiol levels (Barth et al., 2016; Lisofsky et al., 2015). Our results also harmonize well with the neurotrophic and neuroprotective effects of perimenopausal hormone replacement therapy (Albert et al., 2017; Lord et al., 2008; Sohrabji, 2005), which act in concert with serotonin (Amin et al., 2006; Epperson et al., 2012). Such potentially neuroprotective effects may be associated with the antidepressant properties estradiol has during perimenopause (Gordon et al., 2018, 2016a, 2016b). On the other hand, oral contraceptive use, which is a non-dynamic state of endogenous ovarian hormone suppression combined with synthetic steroids, appears to be associated with increased hippocampal volumes (Pletzer, 2019; Pletzer et al., 2019, 2010). Also, hippocampal volume is *decreased* during long-term periods with high female sex-steroid hormone levels, such as pregnancy and puberty (Cameron, 2004; Carmona et al., 2019; Hoekzema et al., 2017; Mills et al., 2016; Sisk and Zehr, 2005). However, this decrease is associated with a functional and structural restructuring of the brain that may help the healthy brain to perform better under new life circumstances, e.g. prepare the maternal brain for motherhood (Carmona et al., 2019; Hoekzema et al., 2017; Pawluski et al., 2016, 2009). In other words, it seems like differences in length, magnitude and timing of an estradiol stimulation or fluctuations critically determine the effects on brain structure and function (Lord et al., 2008; Rehbein et al., 2021). Although preliminary, our data points to loss of neuroprotection, at least transiently, in response to abrupt estradiol decreases, which is in line with studies of hippocampal volume changes over the menstrual cycle or during menopausal transition and may be associated with the antidepressant properties of estradiol in perimenopause.

Serotonin transporter (SERT) availability and hippocampal volume

We found no evidence for a moderation effect (i.e., an interaction effect), as was observed for the previously published depressogenic effects of change in SERT and change in estradiol concentrations (Frokjaer et al., 2015). However, SERT binding at baseline contributed to the model for hippocampal volume changes. Intriguingly, baseline SERT binding was negatively associated with hippocampal volume at baseline, but positively associated with the *change* in hippocampal volume from baseline. Thus, even though low baseline SERT binding may be associated with a larger hippocampal volume in general, it may also be associated with larger susceptibility for

decreased hippocampal volume across hormonal transitions. While the interpretation of the direction is not straightforward, it corresponds well with the neuroplastic properties of the serotonin system, including SERT (Alenina and Klempin, 2015; Kraus et al., 2017). We speculate that this increased hippocampal sensitivity to (loss of) trophic stimuli may be driven by changes in gene transcription, as is the case for vulnerability to depressive symptoms in relation to sex-hormone transitions (Mehta et al., 2019).

Overall, our study suggests that brain SERT and estradiol fluctuations may both be associated with hippocampal volume changes.

Hippocampal volume and the emergence of depressive symptoms

Contrary to our expectations, hippocampal volume changes within the GnRHa group were not associated with depressive symptoms (Frokjaer et al., 2015). Additional mediations analyses supported this. Thus, our results do not indicate that the reported changes in hippocampal volume play a role in the underlying mechanisms of GnRHa induced despressogenic changes in hippocampal functional connectivity, in this cohort (Fisher et al., 2017). Additionally, our results do not directly align with the evidence for an association between depressive symptoms in clinical populations and decreased hippocampal volume (McKinnon et al., 2009; Schmaal et al., 2016; Videbech and Ravnkilde, 2004). However, the women in the GnRHa group only had subclinical depressive symptoms for a short period of time, and may not have experienced the full range of neurobiological changes related to a manifest depressive episode. Although we previously published a SERT-by-estradiol interaction effect on depressive symptoms in this cohort, the effects of SERT on hippocampal volume were not associated with the emergence of depressive symptoms (Frokjaer et al., 2015). Thus, it seems like SERT affects mood and hippocampal volume through different mechanisms or mechanisms work at different speeds.

Thus, our data suggest that hippocampal volume changes in response to short term sex-hormone fluctuations are well tolerated in the female brain and are not strongly associated with depressive symptoms induced by hormonal transitions, at least in healthy low-risk women.

Methodological considerations

The primary strength of this study is the longitudinal nature, a well-defined pharmacological intervention, and a placebo-controlled design, which directly enables us to model brain signatures of a biphasic ovarian hormone fluctuation. However, the study has several limitations and should be considered preliminary. First, our study, including sample size selection, was mainly designed to answer questions related to SERT, estradiol and mood, as emphasized by the subset of women who had a suboptimal timing of their MRI (i.e., > 4 days apart from PET scan or blood samples). Second, the sample size and the mentally robust nature of the study population, reduced our ability to detect more discrete effects due to power limitations. Third, acute changes in hippocampal volume in healthy individuals are of a small magnitude and thus vulnerable to measurement noise, including image analysis methods (Gronenschild et al., 2012; Kong et al., 2022). Larger samples and/or higher image resolution, i.e., in 7 Tesla fields, may to some extent alleviate this problem in future studies. However, even though the magnitude of the observed volume changes were small, they are similar to those that occur over the menstrual cycle (Lisofsky et al., 2015) and during depressive episodes (Schmaal et al., 2016), which suggests that the observed effects are of a reasonable size. Finally, we did *not* adjust our results for multiple comparisons. This was mainly because the study was driven by few *a priori* hypotheses, which were supplemented with robustness analyses. However, we also acknowledge the preliminary nature of the study, where we seek to uncover potential patterns of associations between weak signals, within a study design optimized for other research questions. Nevertheless, we believe that such an approach is justified and valuable for hypothesis generation, provided that it is evaluated in future studies, as this type of dataset is rare. Larger studies of hippocampal volume changes in response to pharmacological sex-hormone manipulation and studies in high-risk for depression individuals, across hormonal states, or groups with high hormone sensitivity are warranted.

Conclusions

In summary, we did not find a significant decrease in hippocampal volume in response to GnRHa relative to placebo. Within the GnRHa group we found no evidence that hippocampal volume mediated the association between estradiol changes and the emergence of depressive symptoms.

However, change in hippocampal volume was related to the magnitude of the GnRHa-induced estradiol decrease, when controlled for baseline SERT binding, but not linked to the emergence of depressive symptoms in this cohort. This suggests that both estradiol dynamics and serotonergic brain architecture may be important for hippocampal plasticity and neuroprotection. However, we acknowledge that this study has a number of limitations and should be considered preliminary. If replicated, ideally in larger datasets and/or in high-risk populations, our data indicate that estradiol fluctuations may be associated with hippocampal plasticity, and that abrupt hormonal withdrawal may compromise neuroprotection, but not necessarily in a depressogenic way.

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References:

Abbafati, C., Abbas, K.M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K.H., Abolhassani, H., Aboyans, V., Abreu, L.G., Abrigo, M.R.M., Abualhasan, A., Abu-Raddad, L.J., Abushouk, A.I., Adabi, M., Adekanmbi, V., Adeoye, A.M., Adetokunboh, O.O., Adham, D., Advani, S.M., Afshin, A., Agarwal, G., Aghamir, S.M.K., Agrawal, A., Ahmad, T., Ahmadi, K., Ahmadi, M., Ahmadieh, H., Ahmed, M.B., Akalu, T.Y., Akinyemi, R.O., Akinyemiju, T., Akombi, B., Akunna, C.J., Alahdab, F., Al-Aly, Z., Alam, K., Alam, S., Alam, T., Alanezi, F.M., Alanzi, T.M., Alemu, B.W., Alhabib, K.F., Ali, M., Ali, S., Alicandro, G., Alinia, C., Alipour, V., Alizade, H., Aljunid, S.M., Alla, F., Allebeck, P., Almasi-Hashiani, A., Al-Mekhlafi, H.M., Alonso, J., Altirkawi, K.A., Amini-Rarani, M., Amiri, F., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Anderson, J.A., Andrei, C.L., Andrei, T., Angus, C., Anjomshoa, M., Ansari, F., Ansari-Moghaddam, A., Antonazzo, I.C., Antonio, C.A.T., Antony, C.M., Antriyandarti, E., Anvari, D., Anwer, R., Appiah, S.C.Y., Arabloo, J., Arab-Zozani, M., Aravkin, A.Y., Ariani, F., Armoon, B., Ärnlöv, J., Arzani, A., Asadi-Aliabadi, M., Asadi-Pooya, A.A., Ashbaugh, C., Assmus, M., Atafar, Z., Atnafu, D.D., Atout, M.M. d. W., Ausloos, F., Ausloos, M., Ayala Quintanilla, B.P., Ayano, G., Ayanore, M.A., Azari, S., Azarian, G., Azene, Z.N., Badawi, A., Badiye, A.D., Bahrami, M.A., Bakhshaei, M.H., Bakhtiari, A., Bakkannavar, S.M., Baldasseroni, A., Ball, K., Ballew, S.H., Balzi, D., Banach, M., Banerjee, S.K., Bante, A.B., Baraki, A.G., Barker-Collo, S.L., Bärnighausen, T.W., Barrero, L.H., Barthelemy, C.M., Barua, L., Basu, S., Baune, B.T., Bayati, M., Becker, J.S., Bedi, N., Beghi, E., Béjot, Y., Bell, M.L., Bennitt, F.B., Bensenor, I.M., Berhe, K., Berman, A.E., Bhagavathula, A.S., Bhageerathy, R., Bhala, N., Bhandari, D., Bhattacharyya, K., Bhutta, Z.A., Bijani, A., Bikbov, B., Bin Sayeed, M.S., Biondi, A., Birihane, B.M., Bisignano, C., Biswas, R.K., Bitew, H., Bohlouli, S., Bohluli, M., Boon-Dooley, A.S., Borges, G., Borzì, A.M., Borzouei, S., Bosetti, C., Boufous, S., Braithwaite, D., Brauer, M., Breitborde, N.J.K., Breitner, S., Brenner, H., Briant, P.S., Briko, A.N., Briko, N.I., Britton, G.B., Bryazka, D., Bumgarner, B.R., Burkart, K., Burnett, R.T., Burugina Nagaraja, S., Butt, Z.A., Caetano Dos Santos, F.L., Cahill, L.E., Cámera, L.A., Campos-Nonato, I.R., Cárdenas, R., Carreras, G., Carrero, J.J., Carvalho, F., Castaldelli-Maia, J.M., Castañeda-Orjuela, C.A., Castelpietra, G., Castro, F., Causey, K., Cederroth, C.R., Cercy, K.M., Cerin, E., Chandan, J.S.,

Chang, K.L., Charlson, F.J., Chattu, V.K., Chaturvedi, S., Cherbuin, N., Chimed-Ochir, O., Cho, D.Y., Choi, J.Y.J., Christensen, H., Chu, D.T., Chung, M.T., Chung, S.C., Cicuttini, F.M., Ciobanu, L.G., Cirillo, M., Classen, T.K.D., Cohen, A.J., Compton, K., Cooper, O.R., Costa, V.M., Cousin, E., Cowden, R.G., Cross, D.H., Cruz, J.A., Dahlawi, S.M.A., Damasceno, A.A.M., Damiani, G., Dandona, L., Dandona, R., Dangel, W.J., Danielsson, A.K., Dargan, P.I., Darwesh, A.M., Daryani, A., Das, J.K., Das Gupta, Rajat, das Neves, J., Dávila-Cervantes, C.A., Davitoiu, D.V., De Leo, D., Degenhardt, L., DeLang, M., Dellavalle, R.P., Demeke, F.M., Demoz, G.T., Demsie, D.G., Denova-Gutiérrez, E., Dervenis, N., Dhungana, G.P., Dianatinasab, M., Dias da Silva, D., Diaz, D., Dibaji Forooshani, Z.S., Djalalinia, S., Do, H.T., Dokova, K., Dorostkar, F., Doshmangir, L., Driscoll, T.R., Duncan, B.B., Duraes, A.R., Eagan, A.W., Edvardsson, D., El Nahas, N., El Sayed, I., El Tantawi, M., Elbarazi, I., Elgendy, I.Y., El-Jaafary, S.I., Elyazar, I.R.F., Emmons-Bell, S., Erskine, H.E., Eskandarieh, S., Esmaeilnejad, S., Esteghamati, A., Estep, K., Etemadi, A., Etisso, A.E., Fanzo, J., Farahmand, M., Fareed, M., Faridnia, R., Farioli, A., Faro, A., Faruque, M., Farzadfar, F., Fattahi, N., Fazlzadeh, M., Feigin, V.L., Feldman, R., Fereshtehnejad, S.M., Fernandes, E., Ferrara, G., Ferrari, A.J., Ferreira, M.L., Filip, I., Fischer, F., Fisher, J.L., Flor, L.S., Foigt, N.A., Folayan, M.O., Fomenkov, A.A., Force, L.M., Foroutan, M., Franklin, R.C., Freitas, M., Fu, W., Fukumoto, T., Furtado, J.M., Gad, M.M., Gakidou, E., Gallus, S., Garcia-Basteiro, A.L., Gardner, W.M., Geberemariyam, B.S., Ayalew Gebreslassie, A.A.A., Geremew, A., Gershberg Hayoon, A., Gething, P.W., Ghadimi, M., Ghadiri, K., Ghaffarifar, F., Ghafourifard, M., Ghamari, F., Ghashghaee, A., Ghiasvand, H., Ghith, N., Gholamian, A., Ghosh, R., Gill, P.S., Ginindza, T.G., Giussani, G., Gnedovskaya, E. V., Goharinezhad, S., Gopalani, S.V., Gorini, G., Goudarzi, H., Goulart, A.C., Greaves, F., Grivna, M., Grosso, G., Gubari, M.I.M., Gugnani, H.C., Guimarães, R.A., Guled, R.A., Guo, G., Guo, Y., Gupta, Rajeev, Gupta, T., Haddock, B., Hafezi-Nejad, N., Hafiz, A., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hall, B.J., Halvaei, I., Hamadeh, R.R., Hamidi, S., Hammer, M.S., Hankey, G.J., Haririan, H., Haro, J.M., Hasaballah, A.I., Hasan, M.M., Hasanpoor, E., Hashi, A., Hassanipour, S., Hassankhani, H., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Heidari-Soureshjani, R., Henrikson, H.J., Herbert, M.E., Herteliu, C., Heydarpour, F., Hird, T.R., Hoek, H.W., Holla, R., Hoogar, P., Hosgood, H.D., Hossain, N., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Hsairi, M., Hsieh, V.C.R., Hu, G., Hu, K., Huda, T.M., Humayun, A., Huynh, C.K., Hwang, B.F., Iannucci, V.C., Ibitoye, S.E.,

Ikeda, N., Ikuta, K.S., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Inbaraj, L.R., Ippolito, H., Iqbal, U., Irvani, S.S.N., Irvine, C.M.S., Islam, M.M., Islam, S.M.S., Iso, H., Ivers, R.Q., Iwu, C.C.D., Iwu, C.J., Iyamu, I.O., Jaafari, J., Jacobsen, K.H., Jafari, H., Jafarinia, M., Jahani, M.A., Jakovljevic, M., Jalilian, F., James, S.L., Janjani, H., Javaheri, T., Javidnia, J., Jeemon, P., Jenabi, E., Jha, R.P., Jha, V., Ji, J.S., Johansson, L., John, O., John-Akinola, Y.O., Johnson, C.O., Jonas, J.B., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kabir, Z., Kalani, H., Kalani, R., Kalankesh, L.R., Kalhor, R., Kanchan, T., Kapoor, N., Matin, B.K., Karch, A., Karim, M.A., Kassa, G.M., Katikireddi, S.V., Kayode, G.A., Kazemi Karyani, A., Keiyoro, P.N., Keller, C., Kemmer, L., Kendrick, P.J., Khalid, N., Khammarnia, M., Khan, E.A., Khan, M., Khatab, K., Khater, M.M., Khatib, M.N., Khayamzadeh, M., Khazaei, S., Kieling, C., Kim, Y.J., Kimokoti, R.W., Kisa, A., Kisa, S., Kivimäki, M., Knibbs, L.D., Knudsen, A.K.S., Kocarnik, J.M., Kochhar, S., Kopec, J.A., Korshunov, V.A., Koul, P.A., Koyanagi, A., Kraemer, M.U.G., Krishan, K., Krohn, K.J., Kromhout, H., Kuate Defo, B., Kumar, G.A., Kumar, V., Kurmi, O.P., Kusuma, D., La Vecchia, C., Lacey, B., Lal, D.K., Lalloo, R., Lallukka, T., Lami, F.H., Landires, I., Lang, J.J., Langan, S.M., Larsson, A.O., Lasrado, S., Lauriola, P., Lazarus, J. V., Lee, P.H., Lee, S.W.H., Legrand, K.E., Leigh, J., Leonardi, M., Lescinsky, H., Leung, J., Levi, M., Li, S., Lim, L.L., Linn, S., Liu, Shiwei, Liu, Simin, Liu, Y., Lo, J., Lopez, A.D., Lopez, J.C.F., Lopukhov, P.D., Lorkowski, S., Lotufo, P.A., Lu, A., Lugo, A., Maddison, E.R., Mahasha, P.W., Mahdavi, M.M., Mahmoudi, M., Majeed, A., Maleki, A., Maleki, S., Malekzadeh, R., Malta, D.C., Mamun, A.A., Manda, A.L., Manguerra, H., Mansour-Ghanaei, F., Mansouri, B., Mansournia, M.A., Mantilla Herrera, A.M., Maravilla, J.C., Marks, A., Martin, R. V., Martini, S., Martins-Melo, F.R., Masaka, A., Masoumi, S.Z., Mathur, M.R., Matsushita, K., Maulik, P.K., McAlinden, C., McGrath, J.J., McKee, M., Mehndiratta, M.M., Mehri, F., Mehta, K.M., Memish, Z.A., Mendoza, W., Menezes, R.G., Mengesha, E.W., Mereke, A., Mereta, S.T., Meretoja, A., Meretoja, T.J., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mills, E.J., Mini, G.K., Miri, M., Mirica, A., Mirrakhimov, E.M., Mirzaei, H., Mirzaei, M., Mirzaei, R., Mirzaei-Alavijeh, M., Misganaw, A.T., Mithra, P., Moazen, B., Mohammad, D.K., Mohammad, Y., Mohammad Gholi Mezerji, N., Mohammadian-Hafshejani, A., Mohammadifard, N., Mohammadpourhodki, R., Mohammed, A.S., Mohammed, H., Mohammed, J.A., Mohammed, S., Mokdad, A.H., Molokhia, M., Monasta, L., Mooney, M.D., Moradi, G., Moradi, M., Moradi-Lakeh, M., Moradzadeh, R., Moraga, P.,

Morawska, L., Morgado-Da-Costa, J., Morrison, S.D., Mosapour, A., Mosser, J.F., Mouodi, S., Mousavi, S.M., Khaneghah, A.M., Mueller, U.O., Mukhopadhyay, S., Mullany, E.C., Musa, K.I., Muthupandian, S., Nabhan, A.F., Naderi, M., Nagarajan, A.J., Nagel, G., Naghavi, M., Naghshtabrizi, B., Naimzada, M.D., Najafi, F., Nangia, V., Nansseu, J.R., Naserbakht, M., Nayak, V.C., Negoi, I., Ngunjiri, J.W., Nguyen, C.T., Nguyen, H.L.T., Nguyen, M., Nigatu, Y.T., Nikbakhsh, R., Nixon, M.R., Nnaji, C.A., Nomura, S., Norrving, B., Noubiap, J.J., Nowak, C., Nunez-Samudio, V., Oancea, B., Odell, C.M., Ogbo, F.A., Oh, I.H., Okunga, E.W., Oladnabi, M., Olagunju, A.T., Olusanya, B.O., Olusanya, J.O., Omer, M.O., Ong, K.L., Onwujekwe, O.E., Orpana, H.M., Ortiz, A., Osarenotor, O., Osei, F.B., Ostroff, S.M., Otoiu, A., Otstavnov, N., Otstavnov, S.S., Øverland, S., Owolabi, M.O., Mahesh, P.A., Padubidri, J.R., Palladino, R., Panda-Jonas, S., Pandey, A., Parry, C.D.H., Pasovic, M., Pasupula, D.K., Patel, S.K., Pathak, M., Patten, S.B., Patton, G.C., Toroudi, H.P., Peden, A.E., Pennini, A., Pepito, V.C.F., Peprah, E.K., Pereira, D.M., Pesudovs, K., Pham, H.Q., Phillips, M.R., Piccinelli, C., Pilz, T.M., Piradov, M.A., Pirsaheb, M., Plass, D., Polinder, S., Polkinghorne, K.R., Pond, C.D., Postma, M.J., Pourjafar, H., Pourmalek, F., Poznañska, A., Prada, S.I., Prakash, V., Pribadi, D.R.A., Pupillo, E., Syed, Z.Q., Rabiee, M., Rabiee, N., Radfar, A., Rafiee, A., Raggi, A., Rahman, M.A., Rajabpour-Sanati, A., Rajati, F., Rakovac, I., Ram, P., Ramezanzadeh, K., Ranabhat, C.L., Rao, P.C., Rao, S.J., Rashedi, V., Rathi, P., Rawaf, D.L., Rawaf, S., Rawal, L., Rawassizadeh, R., Rawat, R., Razo, C., Redford, S.B., Reiner, R.C., Reitsma, M.B., Remuzzi, G., Renjith, V., Renzaho, A.M.N., Resnikoff, S., Rezaei, Negar, Rezaei, Nima, Rezapour, A., Rhinehart, P.A., Riahi, S.M., Ribeiro, D.C., Ribeiro, D., Rickard, J., Rivera, J.A., Roberts, N.L.S., Rodríguez-Ramírez, S., Roever, L., Ronfani, L., Room, R., Roshandel, G., Roth, G.A., Rothenbacher, D., Rubagotti, E., Rwegerera, G.M., Sabour, S., Sachdev, P.S., Saddik, B., Sadeghi, E., Sadeghi, M., Saeedi, R., Saeedi Moghaddam, S., Safari, Y., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sajadi, S.M., Salam, N., Salamati, P., Salem, H., Salem, M.R., Salimzadeh, H., Salman, O.M., Salomon, J.A., Samad, Z., Samadi Kafil, H., Sambala, E.Z., Samy, A.M., Sanabria, J., Sánchez-Pimienta, T.G., Santomauro, D.F., Santos, I.S., Santos, J.V., Santric-Milicevic, M.M., Saraswathy, S.Y.I., Sarmiento-Suárez, R., Sarrafzadegan, N., Sartorius, B., Sarveazad, A., Sathian, B., Sathish, T., Sattin, D., Saxena, S., Schaeffer, L.E., Schiavolin, S., Schlaich, M.P., Schmidt, M.I., Schutte, A.E., Schwebel, D.C., Schwendicke, F., Senbeta, A.M., Senthilkumaran, S., Sepanlou, S.G., Serdar, B., Serre, M.L.,

Shadid, J., Shafaat, O., Shahabi, S., Shaheen, A.A., Shaikh, M.A., Shalash, A.S., Shams-Beyranvand, M., Shamsizadeh, M., Sharafi, K., Sheikh, A., Sheikhtaheri, A., Shibuya, K., Shield, K.D., Shigematsu, M., Shin, J. Il, Shin, M.J., Shiri, R., Shirkoohi, R., Shuval, K., Siabani, S., Sierpinski, R., Sigfusdottir, I.D., Sigurvinsdottir, R., Silva, J.P., Simpson, K.E., Singh, J.A., Singh, P., Skiadaresi, E., Skou, S.T., Skryabin, V.Y., Smith, E.U.R., Soheili, A., Soltani, S., Soofi, M., Sorensen, R.J.D., Soriano, J.B., Sorrie, M.B., Soshnikov, S., Soyiri, I.N., Spencer, C.N., Spotin, A., Sreeramareddy, C.T., Srinivasan, V., Stanaway, J.D., Stein, C., Stein, D.J., Steiner, C., Stockfelt, L., Stokes, M.A., Straif, K., Stubbs, J.L., Sufiyan, M.B., Suleria, H.A.R., Suliankatchi Abdulkader, R., Sulo, G., Sultan, I., Tabarés-Seisdedos, R., Tabb, K.M., Tabuchi, T., Taherkhani, A., Tajdini, M., Takahashi, K., Takala, J.S., Tamiru, A.T., Taveira, N., Tehrani-Banihashemi, A., Temsah, M.H., Tesema, G.A., Tessema, Z.T., Thurston, G.D., Titova, M.V., Tohidinik, H.R., Tonelli, M., Topor-Madry, R., Topouzis, F., Torre, A.E., Touvier, M., Tovani-Palone, M.R., Tran, B.X., Travillian, R., Tsatsakis, A., Tudor Car, L.T., Tyrovolas, S., Uddin, R., Umeokonkwo, C.D., Unnikrishnan, B., Upadhyay, E., Vacante, M., Valdez, P.R., van Donkelaar, A., Vasankari, T.J., Vasseghian, Y., Veisani, Y., Venketasubramanian, N., Violante, F.S., Vlassov, V., Vollset, S.E., Vos, T., Vukovic, R., Waheed, Y., Wallin, M.T., Wang, Y., Wang, Y.P., Watson, A., Wei, J., Wei, M.Y.W., Weintraub, R.G., Weiss, J., Werdecker, A., West, J.J., Westerman, R., Whisnant, J.L., Whiteford, H.A., Wiens, K.E., Wolfe, C.D.A., Wozniak, S.S., Wu, A.M., Wu, J., Wulf Hanson, S., Xu, G., Xu, R., Yadgir, S., Yahyazadeh Jabbari, S.H., Yamagishi, K., Yaminfirooz, M., Yano, Y., Yaya, S., Yazdi-Feyzabadi, V., Yeheyis, T.Y., Yilgwan, C.S., Yilma, M.T., Yip, P., Yonemoto, N., Younis, M.Z., Younker, T.P., Yousefi, B., Yousefi, Z., Yousefinezhadi, T., Yousuf, A.Y., Yu, C., Yusefzadeh, H., Moghadam, T.Z., Zamani, M., Zamanian, M., Zandian, H., Zastrozhin, M.S., Zhang, Y., Zhang, Z.J., Zhao, J.T., Zhao, X.J.G., Zhao, Y., Zheng, P., Zhou, M., Davletov, K., Ziapour, A., Mondello, S., Lim, S.S., Murray, C.J.L., Wiangkham, T., Amini, S., 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396, 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Albert, K., Hiscox, J., Boyd, B., Dumas, J., Taylor, W., Newhouse, P., 2017. Estrogen enhances hippocampal gray-matter volume in young and older postmenopausal women: a prospective dose-response study. Neurobiol. Aging 56, 1–6.

https://doi.org/10.1016/j.neurobiolaging.2017.03.033

Alenina, N., Klempin, F., 2015. The role of serotonin in adult hippocampal neurogenesis. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2014.07.038

Amin, Z., Gueorguieva, R., Cappiello, A., Czarkowski, K.A., Stiklus, S., Anderson, G.M., Naftolin, F., Epperson, C.N., 2006. Estradiol and tryptophan depletion interact to modulate cognition in menopausal women. Neuropsychopharmacology 31, 2489–2497. https://doi.org/10.1038/sj.npp.1301114

Barth, C., Steele, C.J., Mueller, K., Rekkas, V.P., Arélin, K., Pampel, A., Burmann, I., Kratzsch, J., Villringer, A., Sacher, J., 2016. In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. Sci. Rep. 6, 32833. https://doi.org/10.1038/srep32833

Barth, C., Villringer, A., Sacher, J., 2015. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. Front. Neurosci. 9. https://doi.org/10.3389/fnins.2015.00037

Bethea, C.L., Reddy, A.P., Tokuyama, Y., Henderson, J.A., Lima, F.B., 2009. Protective actions of ovarian hormones in the serotonin system of macaques. Front. Neuroendocrinol. 30, 212–238. https://doi.org/10.1016/j.yfrne.2009.04.003

Cameron, J.L., 2004. Interrelationships between hormones, behavior, and affect during adolescence: Understanding hormonal, physical, and brain changes occurring in association with pubertal activation of the reproductive axis - Introduction to part III. Ann. N. Y. Acad. Sci. 1021, 110–123. https://doi.org/10.1196/annals.1308.012

Carmona, S., Martínez-García, M., Paternina-Die, M., Barba-Müller, E., Wierenga, L.M., Alemán-Gómez, Y., Pretus, C., Marcos-Vidal, L., Beumala, L., Cortizo, R., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J.C., Tobeña, A., Peper, J.S., Crone, E.A., Ballesteros, A., Vilarroya, O., Desco, M., Hoekzema, E., 2019. Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes. Hum. Brain Mapp. 40, 2143–2152. https://doi.org/10.1002/hbm.24513

Eid, R.S., Chaiton, J.A., Lieblich, S.E., Bodnar, T.S., Weinberg, J., Galea, L.A.M., 2019. Early and late effects of maternal experience on hippocampal neurogenesis, microglia, and the circulating cytokine milieu. Neurobiol. Aging 78, 1–17. https://doi.org/10.1016/j.neurobiolaging.2019.01.021

Enneking, V., Leehr, E.J., Dannlowski, U., Redlich, R., 2019. Brain structural effects of treatments for depression and biomarkers of response: A systematic review of neuroimaging studies. Psychol. Med. 50, 187–209. https://doi.org/10.1017/S0033291719003660

Epperson, C.N., Amin, Z., Ruparel, K., Gur, R., Loughead, J., 2012. Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women. Psychoneuroendocrinology 37, 372–382. https://doi.org/10.1016/j.psyneuen.2011.07.007

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole Brain Segmentation. Neuron 33, 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X

Fisher, P.M.D., Larsen, C.B., Beliveau, V., Henningsson, S., Pinborg, A., Holst, K.K., Jensen, P.S., Svarer, C., Siebner, H.R., Knudsen, G.M., Frokjaer, V.G., 2017. Pharmacologically Induced Sex Hormone Fluctuation Effects on Resting-State Functional Connectivity in a Risk Model for Depression: A Randomized Trial. Neuropsychopharmacology 42, 446–453. https://doi.org/10.1038/npp.2016.208

Fraser, M.A., Shaw, M.E., Cherbuin, N., 2015. A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. Neuroimage 112, 364–374. https://doi.org/10.1016/j.neuroimage.2015.03.035

Freeman, E.W., Sammel, M.D., Boorman, D.W., Zhang, R., 2014. Longitudinal pattern of depressive symptoms around natural menopause. JAMA Psychiatry 71, 36–43. https://doi.org/10.1001/jamapsychiatry.2013.2819

Frokjaer, V.G., 2020. Pharmacological sex hormone manipulation as a risk model for depression. J. Neurosci. Res. https://doi.org/10.1002/jnr.24632

Frokjaer, V.G., Erritzoe, D., Juul, A., Nielsen, F.Å., Holst, K., Svarer, C., Madsen, J., Paulson, O.B., Knudsen, G.M., 2010. Endogenous plasma estradiol in healthy men is positively correlated with cerebral cortical serotonin 2A receptor binding. Psychoneuroendocrinology 35, 1311–1320. https://doi.org/10.1016/j.psyneuen.2010.03.002

Frokjaer, V.G., Pinborg, A., Holst, K.K., Overgaard, A., Henningsson, S., Heede, M., Larsen, E.C., Jensen, P.S., Agn, M., Nielsen, A.P., Stenbæk, D.S., Da Cunha-Bang, S., Lehel, S., Siebner, H.R., Mikkelsen, J.D., Svarer, C., Knudsen, G.M., 2015. Role of serotonin transporter changes in

depressive responses to sex-steroid hormone manipulation: A positron emission tomography study. Biol. Psychiatry 78, 534–543. https://doi.org/10.1016/j.biopsych.2015.04.015

Gordon, J.L., Eisenlohr-Moul, T.A., Rubinow, D.R., Schrubbe, L., Girdler, S.S., 2016a. Naturally occurring changes in estradiol concentrations in the menopause transition predict morning cortisol and negative mood in perimenopausal depression. Clin. Psychol. Sci. 4, 919–935. https://doi.org/10.1177/2167702616647924

Gordon, J.L., Rubinow, D.R., Eisenlohr-Moul, T.A., Leserman, J., Girdler, S.S., 2016b. Estradiol variability, stressful life events, and the emergence of depressive symptomatology during the menopausal transition. Menopause 23, 257–266. https://doi.org/10.1097/GME.0000000000000528

Gordon, J.L., Rubinow, D.R., Eisenlohr-Moul, T.A., Xia, K., Schmidt, P.J., Girdler, S.S., 2018. Efficacy of transdermal estradiol and micronized progesterone in the prevention of depressive symptoms in the menopause transition: A randomized clinical trial. JAMA Psychiatry 75, 149–157. https://doi.org/10.1001/jamapsychiatry.2017.3998

Gould, E., Tanapat, P., Rydel, T., Hastings, N., 2000. Regulation of hippocampal neurogenesis in adulthood. Biol. Psychiatry 48, 715–720. https://doi.org/10.1016/S0006-3223(00)01021-0

Gronenschild, E.H.B.M., Habets, P., Jacobs, H.I.L., Mengelers, R., Rozendaal, N., van Os, J., Marcelis, M., 2012. The effects of FreeSurfer version, workstation type, and Macintosh operating system version on anatomical volume and cortical thickness measurements. PLoS One 7. https://doi.org/10.1371/journal.pone.0038234

Guintivano, J., Arad, M., Gould, T.D., Payne, J.L., Kaminsky, Z.A., 2014. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. Mol. Psychiatry 19, 560–567. https://doi.org/10.1038/mp.2013.62

HAMILTON, M., 1967. Development of a Rating Scale for Primary Depressive Illness. Br. J. Soc. Clin. Psychol. 6, 278–296. https://doi.org/10.1111/j.2044-8260.1967.tb00530.x

Henningsson, S., Madsen, K.H., Pinborg, A., Heede, M., Knudsen, G.M., Siebner, H.R., Frokjaer, V.G., 2015. Role of emotional processing in depressive responses to sex-hormone manipulation: A pharmacological fMRI study. Transl. Psychiatry 5, e688–e688. https://doi.org/10.1038/tp.2015.184

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J.C.,

Tobeña, A., Desco, M., Crone, E.A., Ballesteros, A., Carmona, S., Vilarroya, O., 2017. Pregnancy leads to long-lasting changes in human brain structure. Nat. Neurosci. 20, 287–296. https://doi.org/10.1038/nn.4458

Holst, K.K., Budtz-Jørgensen, E., 2012. Linear latent variable models: the lava-package. Comput. Stat. 2012 284 28, 1385–1452. https://doi.org/10.1007/S00180-012-0344-Y

Kong, X.Z., Francks, C., Kong, X.Z., Mathias, S.R., Guadalupe, T., Abé, C., Agartz, I., Akudjedu, T.N., Aleman, A., Alhusaini, S., Allen, N.B., Ames, D., Andreassen, O.A., Vasquez, A.A., Armstrong, N.J., Asherson, P., Bergo, F., Bastin, M.E., Batalla, A., Bauer, J., Baune, B.T., Baur-Streubel, R., Biederman, J., Blaine, S.K., Boedhoe, P., Bøen, E., Bose, A., Bralten, J., Brandeis, D., Brem, S., Brodaty, H., Yüksel, D., Brooks, S.J., Buitelaar, J., Bürger, C., Bülow, R., Calhoun, V., Calvo, A., Canales-Rodríguez, E.J., Cannon, D.M., Caparelli, E.C., Castellanos, F.X., Cendes, F., Chaim-Avancini, T.M., Chantiluke, K., Chen, Q. lin, Chen, X., Cheng, Y., Christakou, A., Clark, V.P., Coghill, D., Connolly, C.G., Conzelmann, A., Córdova-Palomera, A., Cousijn, J., Crow, T., Cubillo, A., Dannlowski, U., de Bruttopilo, S.A., de Zeeuw, P., Deary, I.J., Demeter, D. V., Di Martino, A., Dickie, E.W., Dietsche, B., Doan, N.T., Doherty, C.P., Doyle, A., Durston, S., Earl, E., Ehrlich, S., Ekman, C.J., Elvsåshagen, T., Epstein, J.N., Fair, D.A., Faraone, S. V., Fernández, G., Flint, C., Filho, G.B., Förster, K., Fouche, J.P., Foxe, J.J., Frodl, T., Fuentes-Claramonte, P., Fullerton, J.M., Garavan, H., do Santos Garcia, D., Gotlib, I.H., Goudriaan, A.E., Grabe, H.J., Groenewold, N.A., Grotegerd, D., Gruber, O., Gurholt, T., Haavik, J., Hahn, T., Hansell, N.K., Harris, M.A., Hartman, C.A., del Carmen Valdés Hernández, M., Heslenfeld, D., Hester, R., Hibar, D.P., Ho, B.C., Ho, T.C., Hoekstra, P.J., van Holst, R.J., Hoogman, M., Høvik, M.F., Howells, F.M., Hugdahl, K., Huyser, C., Ingvar, M., Ishikawa, A., James, A., Jahanshad, N., Jernigan, T.L., Jönsson, E.G., Kaleda, V., Kelly, C., Kerich, M., Keshavan, M.S., Khadka, S., Kircher, T., Kohls, G., Konrad, K., Korucuoglu, O., Krämer, B., Krug, A., Kuntsi, J., Kwon, J.S., Lambregts-Rommelse, N., Landén, M., Lázaro, L., Lebedeva, I., Lenroot, R., Lesch, K.P., Li, Q., Lim, K.O., Liu, J., Lochner, C., London, E.D., Lorenzetti, V., Luciano, M., Luijten, M., Lundervold, A.J., Mackey, S., MacMaster, F.P., Maingault, S., Malpas, C.B., Malt, U.F., Mataix-Cols, D., Martin-Santos, R., Mayer, A.R., McCarthy, H., Medland, S., Metha, M., Mitchell, P.B., Mueller, B.A., Maniega, S.M., Mazoyer, B., McDonald, C., McLellan, Q., McMahon, K.L., McPhilemy, G., Momenan, R., Morales, A.M., Narayanaswamy, J.C., Moreira, J.C.V., Nerland, S., Nestor, L.,

Newman, E., Nigg, J.T., Nordvik, J.E., Novotny, S., Weiss, E.O., O'Gorman, R.L., Oosterlaan, J., Oranje, B., Orr, C., Overs, B., Paloyelis, Y., Pauli, P., Paulus, M., Plessen, K.J., von Polier, G.G., Pomarol-Clotet, E., Portella, M.J., Qiu, J., Radua, J., Ramos-Quiroga, J.A., Reddy, Y.C.J., Reif, A., Roberts, G., Rosa, P., Rubia, K., Sacchet, M.D., Sachdev, P.S., Salvador, R., Schmaal, L., Schulte-Rüther, M., Schweren, L., Seitz, J., Serpa, M.H., Shaw, P., Shumskaya, E., Silk, T.J., Simmons, A.N., Simulionyte, E., Sinha, R., Sjoerds, Z., Smelror, R.E., Soliva, J.C., Solowij, N., Souza-Duran, F.L., Sponheim, S.R., Stein, D.J., Stein, E.A., Stevens, M., Strike, L.T., Sudre, G., Sui, J., Tamm, L., Temmingh, H.S., Thoma, R.J., Tomyshev, A., Tronchin, G., Turner, J., Uhlmann, A., van Erp, T.G.M., van den Heuvel, O.A., van der Meer, D., van Eijk, L., Vance, A., Veer, I.M., Veltman, D.J., Venkatasubramanian, G., Vilarroya, O., Vives-Gilabert, Y., Voineskos, A.N., Völzke, H., Vuletic, D., Walitza, S., Walter, H., Walton, E., Wardlaw, J.M., Wen, W., Westlye, L.T., Whelan, C.D., White, T., Wiers, R.W., Wright, M.J., Wittfeld, K., Yang, T.T., Yasuda, C.L., Yoncheva, Y., Yücel, M., Yun, J.Y., Zanetti, M.V., Zhen, Z., Zhu, X. xing, Ziegler, G.C., de Zubicaray, G.I., Zwiers, M., Project, K.S., Glahn, D.C., Crivello, F., Fisher, S.E., Thompson, P.M., Francks, C., Farde, L., Flyckt, L., Engberg, G., Erhardt, S., Fatouros-Bergman, H., Cervenka, S., Schwieler, L., Piehl, F., Agartz, I., Collste, K., Victorsson, P., Malmqvist, A., Hedberg, M., Orhan, F., Sellgren, C., 2022. Reproducibility in the absence of selective reporting: An illustration from large-scale brain asymmetry research. Hum. Brain Mapp. 43, 244–254. https://doi.org/10.1002/hbm.25154

Kraus, C., Castrén, E., Kasper, S., Lanzenberger, R., 2017. Serotonin and neuroplasticity – Links between molecular, functional and structural pathophysiology in depression. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2017.03.007

Kuehner, C., 2017. Why is depression more common among women than among men? The Lancet Psychiatry 4, 146–158. https://doi.org/10.1016/S2215-0366(16)30263-2

Lisofsky, N., Mårtensson, J., Eckert, A., Lindenberger, U., Gallinat, J., Kühn, S., 2015. Hippocampal volume and functional connectivity changes during the female menstrual cycle. Neuroimage 118, 154–162. https://doi.org/10.1016/j.neuroimage.2015.06.012

Lokuge, S., Frey, B.N., Foster, J.A., Soares, C.N., Steiner, M., 2011. Depression in women: Windows of vulnerability and new insights into the link between estrogen and serotonin. J. Clin. Psychiatry 72, e1563–e1569. https://doi.org/10.4088/JCP.11com07089

Lord, C., Buss, C., Lupien, S.J., Pruessner, J.C., 2008. Hippocampal volumes are larger in postmenopausal women using estrogen therapy compared to past users, never users and men: A possible window of opportunity effect. Neurobiol. Aging 29, 95–101. https://doi.org/10.1016/j.neurobiolaging.2006.09.001

Macoveanu, J., Henningsson, S., Pinborg, A., Jensen, P., Knudsen, G.M., Frokjaer, V.G., Siebner, H.R., 2016. Sex-Steroid Hormone Manipulation Reduces Brain Response to Reward. Neuropsychopharmacology 41, 1057–1065. https://doi.org/10.1038/npp.2015.236

Martínez-García, M., Paternina-Die, M., Barba-Müller, E., de Blas, D.M., Beumala, L., Cortizo, R., Pozzobon, C., Marcos-Vidal, L., Fernández-Pena, A., Picado, M., Belmonte-Padilla, E., Massó-Rodriguez, A., Ballesteros, A., Desco, M., Vilarroya, Ó., Hoekzema, E., Carmona, S., 2021. Do pregnancy-induced brain changes reverse? The brain of a mother six years after parturition. Brain Sci. 11, 1–14. https://doi.org/10.3390/brainsci11020168

McKinnon, M.C., Yucel, K., Nazarov, A., MacQueen, G.M., 2009. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J. Psychiatry Neurosci. 34, 41–54.

Medina, J., Workman, J.L., 2020. Maternal experience and adult neurogenesis in mammals: Implications for maternal care, cognition, and mental health. J. Neurosci. Res. 98, 1293–1308. https://doi.org/10.1002/jnr.24311

Mehta, D., Newport, D.J., Frishman, G., Kraus, L., Rex-Haffner, M., Ritchie, J.C., Lori, A., Knight, B.T., Stagnaro, E., Ruepp, A., Stowe, Z.N., Binder, E.B., 2014. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Psychol. Med. 44, 2309–2322. https://doi.org/10.1017/S0033291713003231

Mehta, D., Rex-Haffner, M., Sondergaard, H.B., Pinborg, A., Binder, E.B., Frokjaer, V.G., 2019. Evidence for oestrogen sensitivity in perinatal depression: Pharmacological sex hormone manipulation study. Br. J. Psychiatry 215, 519–527. https://doi.org/10.1192/bjp.2018.234

Mills, K.L., Goddings, A.L., Herting, M.M., Meuwese, R., Blakemore, S.J., Crone, E.A., Dahl, R.E., Güroğlu, B., Raznahan, A., Sowell, E.R., Tamnes, C.K., 2016. Structural brain development between childhood and adulthood: Convergence across four longitudinal samples. Neuroimage 141, 273–281. https://doi.org/10.1016/j.neuroimage.2016.07.044

Munk-Olsen, T., Laursen, T.M., Pedersen, C.B., Mors, O., Mortensen, P.B., 2006. New parents and

mental disorders: A population-based register study. J. Am. Med. Assoc. 296, 2582–2589. https://doi.org/10.1001/jama.296.21.2582

Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V, Puri, B.K., Fusi, L., Bydder, G.M., 2002. Change in brain size during and after pregnancy: Study in healthy women and women with preeclampsia. Am. J. Neuroradiol. 23, 19–26.

Pawluski, J.L., Brummelte, S., Barha, C.K., Crozier, T.M., Galea, L.A.M., 2009. Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. Front. Neuroendocrinol. 30, 343–357. https://doi.org/10.1016/j.yfrne.2009.03.007

Pawluski, J.L., Lambert, K.G., Kinsley, C.H., 2016. Neuroplasticity in the maternal hippocampus: Relation to cognition and effects of repeated stress. Horm. Behav. 77, 86–97. https://doi.org/10.1016/j.yhbeh.2015.06.004

Pletzer, B., 2019. Sex hormones and gender role relate to gray matter volumes in sexually dimorphic brain areas. Front. Neurosci. 13. https://doi.org/10.3389/fnins.2019.00592

Pletzer, B., Harris, T., Hidalgo-Lopez, E., 2018. Subcortical structural changes along the menstrual cycle: beyond the hippocampus. Sci. Rep. 8, 16042. https://doi.org/10.1038/s41598-018-34247-4

Pletzer, B., Harris, T.A., Hidalgo-Lopez, E., 2019. Previous contraceptive treatment relates to grey matter volumes in the hippocampus and basal ganglia. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-47446-4

Pletzer, B., Kronbichler, M., Aichhorn, M., Bergmann, J., Ladurner, G., Kerschbaum, H.H., 2010. Menstrual cycle and hormonal contraceptive use modulate human brain structure. Brain Res. 1348, 55–62. https://doi.org/10.1016/j.brainres.2010.06.019

Protopopescu, X., Butler, T., Pan, H., Root, J., Altemus, M., Polanecsky, M., McEwen, B., Silbersweig, D., Stern, E., 2008. Hippocampal structural changes across the menstrual cycle. Hippocampus 18, 985–988. https://doi.org/10.1002/hipo.20468

Rehbein, E., Hornung, J., Sundström Poromaa, I., Derntl, B., 2021. Shaping of the Female Human Brain by Sex Hormones: A Review. Neuroendocrinology 111, 183–206. https://doi.org/10.1159/000507083

Schmaal, L., Veltman, D.J., Van Erp, T.G.M., Smann, P.G., Frodl, T., Jahanshad, N., Loehrer, E.,

Tiemeier, H., Hofman, A., Niessen, W.J., Vernooij, M.W., Ikram, M.A., Wittfeld, K., Grabe, H.J., Block, A., Hegenscheid, K., Völzke, H., Hoehn, D., Czisch, M., Lagopoulos, J., Hatton, S.N., Hickie, I.B., Goya-Maldonado, R., Krmer, B., Gruber, O., Couvy-Duchesne, B., Rentera, M.E., Strike, L.T., Mills, N.T., De Zubicaray, G.I., McMahon, K.L., Medland, S.E., Martin, N.G., Gillespie, N.A., Wright, M.J., Hall, G.B., MacQueen, G.M., Frey, E.M., Carballedo, A., Van Velzen, L.S., Van Tol, M.J., Van der Wee, N.J., Veer, I.M., Walter, H., Schnell, K., Schramm, E., Normann, C., Schoepf, D., Konrad, C., Zurowski, B., Nickson, T., McIntosh, A.M., Papmeyer, M., Whalley, H.C., Sussmann, J.E., Godlewska, B.R., Cowen, P.J., Fischer, F.H., Rose, M., Penninx, B.W.J.H., Thompson, P.M., Hibar, D.P., 2016. Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. Mol. Psychiatry 21, 806–812. https://doi.org/10.1038/mp.2015.69

Sederevičius, D., Vidal-Piñeiro, D., Sørensen, Ø., van Leemput, K., Iglesias, J.E., Dalca, A. V, Greve, D.N., Fischl, B., Bjørnerud, A., Walhovd, K.B., Fjell, A.M., 2021. Reliability and sensitivity of two whole-brain segmentation approaches included in FreeSurfer – ASEG and SAMSEG. Neuroimage 237, 118113. https://doi.org/10.1016/j.neuroimage.2021.118113

Sheppard, P.A.S., Choleris, E., Galea, L.A.M., 2019. Structural plasticity of the hippocampus in response to estrogens in female rodents. Mol. Brain. https://doi.org/10.1186/s13041-019-0442-7

Sisk, C.L., Zehr, J.L., 2005. Pubertal hormones organize the adolescent brain and behavior. Front. Neuroendocrinol. 26, 163–174. https://doi.org/10.1016/j.yfrne.2005.10.003

Sohrabji, F., 2005. Estrogen: A neuroprotective or proinflammatory hormone? Emerging evidence from reproductive aging models. Ann. N. Y. Acad. Sci. 1052, 75–90. https://doi.org/10.1196/annals.1347.006

Thomas, E.J., Jenkins, J., Lenton, E.A., Cooke, I.D., 1986. Endocrine effects of goserelin, a new depot luteinising hormone releasing hormone agonist. Br. Med. J. (Clin. Res. Ed). 293, 1407–1408. https://doi.org/10.1136/bmj.293.6559.1407-a

Videbech, P., Ravnkilde, B., 2004. Hippocampal volume and depression: A meta-analysis of MRI studies. Am. J. Psychiatry. https://doi.org/10.1176/appi.ajp.161.11.1957

Welch, B.L., 1947. THE GENERALIZATION OF 'STUDENT'S' PROBLEM WHEN SEVERAL DIFFERENT POPULATION VARLANCES ARE INVOLVED. Biometrika 34, 28–35.

https://doi.org/10.1093/biomet/34.1-2.28

Willner, P., Scheel-Krüger, J., Belzung, C., 2013. The neurobiology of depression and antidepressant action. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2012.12.007

Woolley, C., McEwen, B., 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J. Neurosci. 12, 2549. https://doi.org/10.1523/JNEUROSCI.12-07-02549.1992

Yagi, S., Galea, L.A.M., 2019. Sex differences in hippocampal cognition and neurogenesis. Neuropsychopharmacology. https://doi.org/10.1038/s41386-018-0208-4

Yankova, M., Hart, S.A., Woolley, C.S., 2001. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: A serial electron-microscopic study. Proc. Natl. Acad. Sci. U. S. A. 98, 3525–3530. https://doi.org/10.1073/pnas.051624598

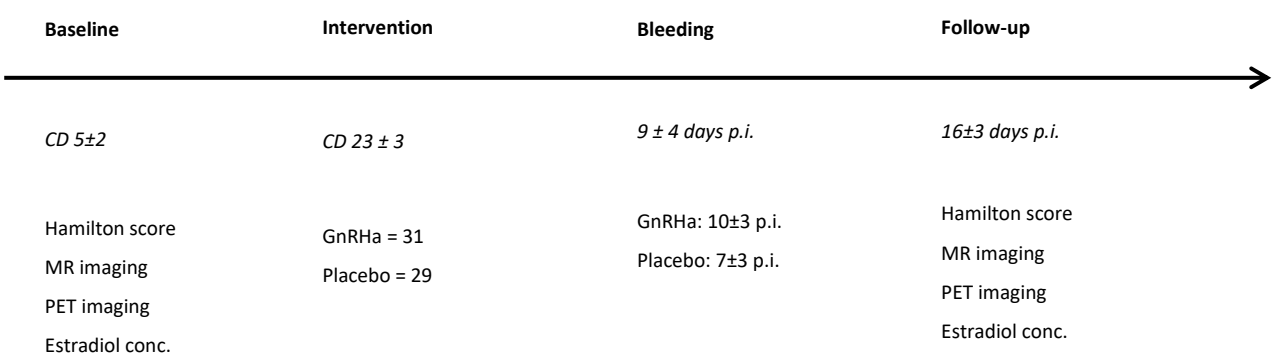
Tables and figures

Table 1. Demographics, hippocampal volume and estradiol concentrations in the GnRHa and placebo group at baseline and follow-up.

	Baseline				Follow-up			
	GnRHa	Placebo	*p-value*	Effect size	GnRHa	Placebo	*p-value*	Effect size
Age *Mean±SD*	23.3±3.3	25.4±5	0.11[a]	0.43[c]	_	_	-	-
5-HTTLPR genotype LALA / S-carrier	10/21	10/19	1[b]	1.1 [0.33;3.70][d]	_	_	-	-
Mean hippocampal volume in mm3 *Mean±SD*	4282±364	4164±374	0.22[a]	0.32[c]	4256±319	4177±390	0.39[a]	0.22[c]
Estradiol concentration in nmol/L *Mean±SD*	0.19±0.09	0.19±0.11	0.96[a]	0.01[c]	0.07±0.03	0.36±0.23	< 0.001[a]	1.62[c]
Hamilton score *Mean±SD*	1.16±1.55	1.59±2.23	0.4[a]	0.22[c]	3.23±3.23	1.76±1.79	0.03[a]	0.56[c]

[a]: two-sample t-test
[b]: *Fisher's* exact test
[c]: Cohen's *d*
[d]: Odds ratio with 95% CI

Figure 1. Overview of the study design. Details on the timing of baseline, intervention and follow-up assessments. For cycle days and post intervention days, we report a mean ± SD.



Abbreviations: CD, cycle day, mean±SD; p.i., post intervention, mean±SD; MR: Magnetic resonance; PET, positron emission tomography; conc., concentration in serum.

Figure 2. Baseline associations between hippocampal volume and estradiol. *Baseline estradiol concentration was associated with baseline hippocampal volume only in a model controlled for age and baseline SERT binding (partial R2 =0.11, p = 0.01, residuals are used to visualize hippocampal volume controlled for SERT binding at baseline and age). Solid line represents regression line with 95% CI.*

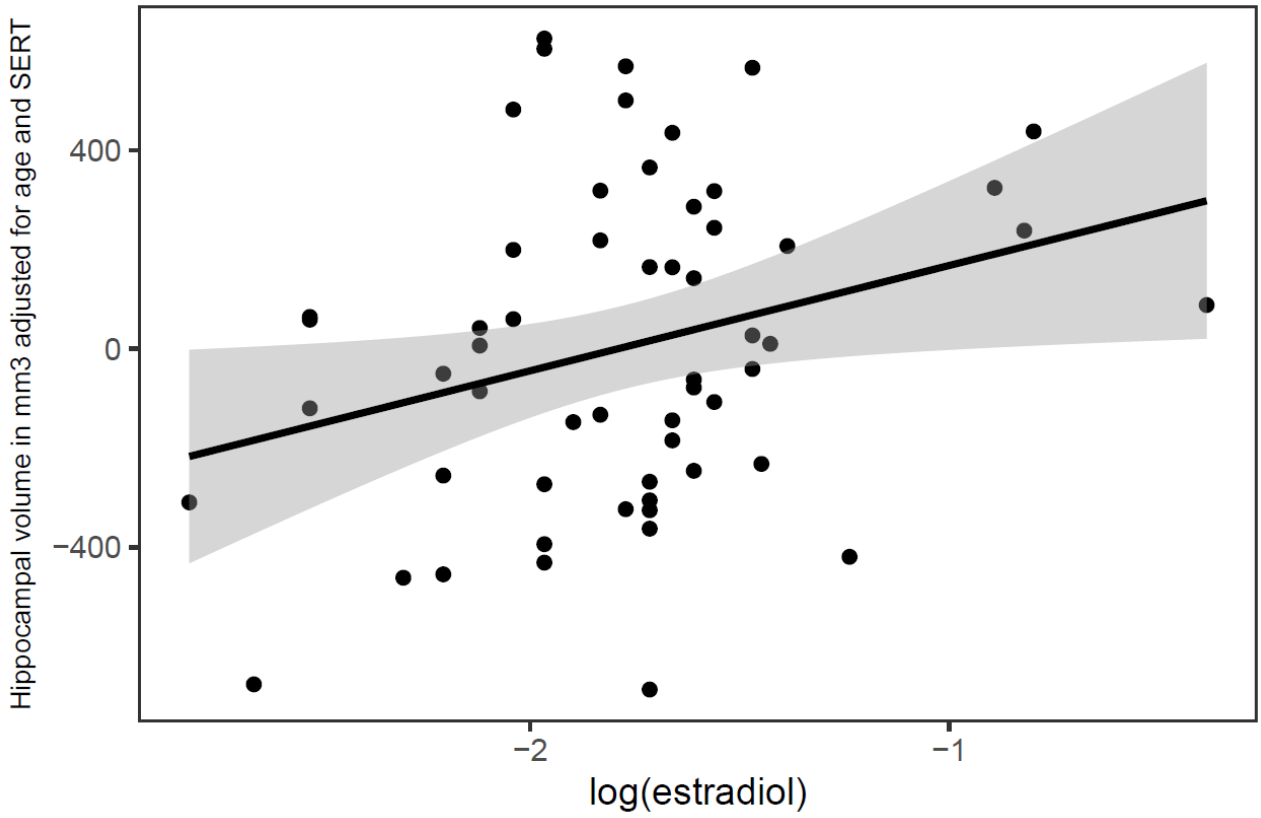

Hippocampal volume in mm3 adjusted for age and SERT
400
0
−400
−2
−1
log(estradiol)

Figure 3. Effect of GnRHa and placebo on change in hippocampal volume

In the GnRHa group, hippocampal volume decreased, as expected, 26.77 mm3 from baseline to follow-up, whereas hippocampal volume in the placebo group increased 12.13 mm3. The mean difference in change of 37.9 mm3, was however not significant (p = 0.14). Black dots represent mean change in hippocampal volume, error bars represent SD.

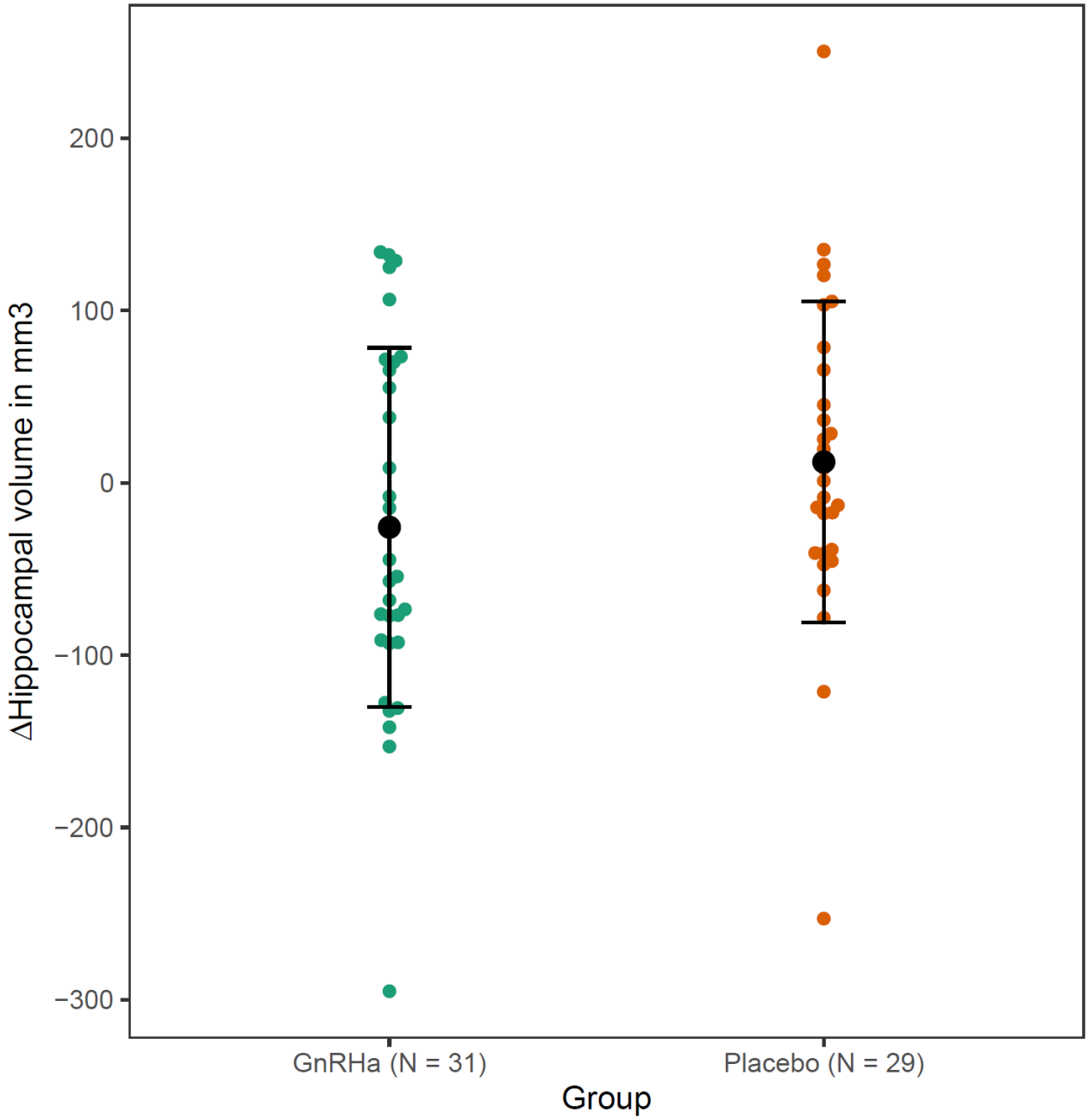


Figure 4. Association between change in hippocampus volume and the emergence of depressive symptoms. *We observed no significant change in hippocampal volume effect on the emergence of depressive symptoms, measured as change in Hamilton depression score (partial R2 = 0.01, p = 0.21). Black line represents regression line with 95% CI.*

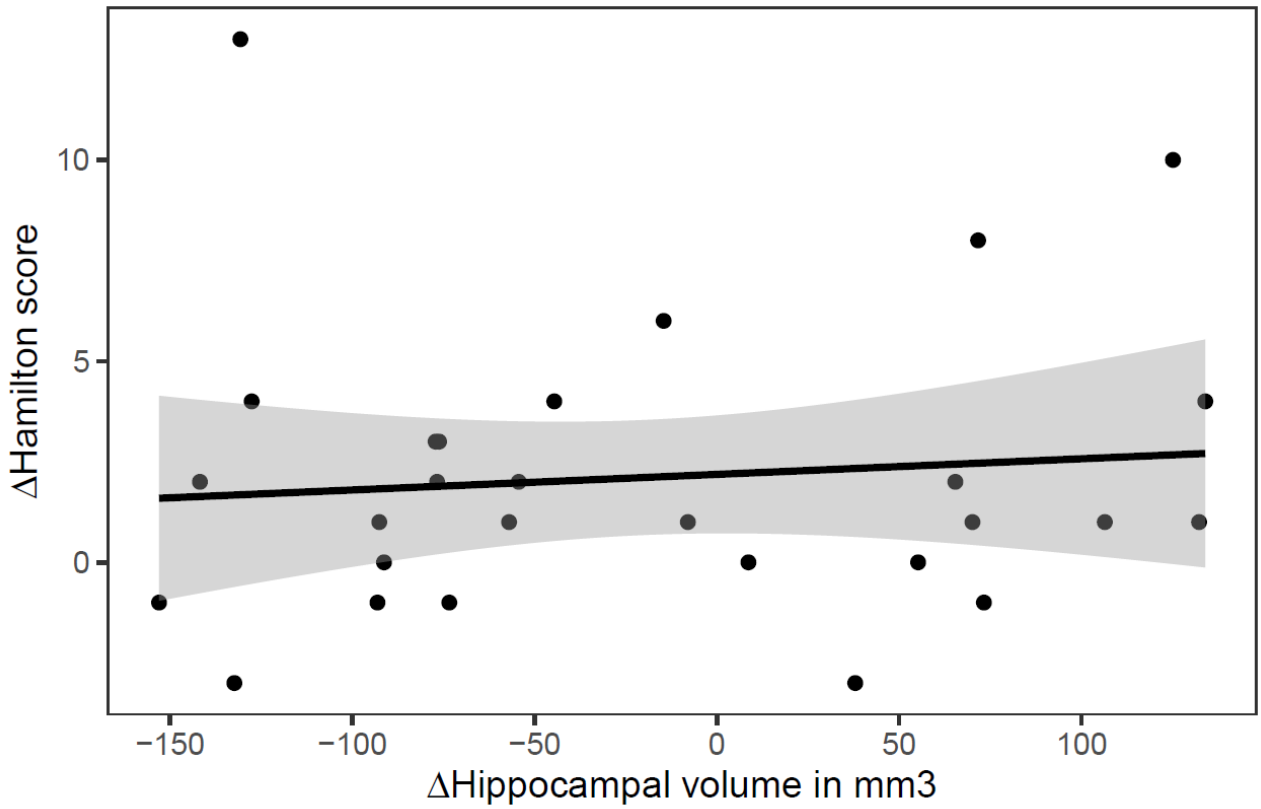
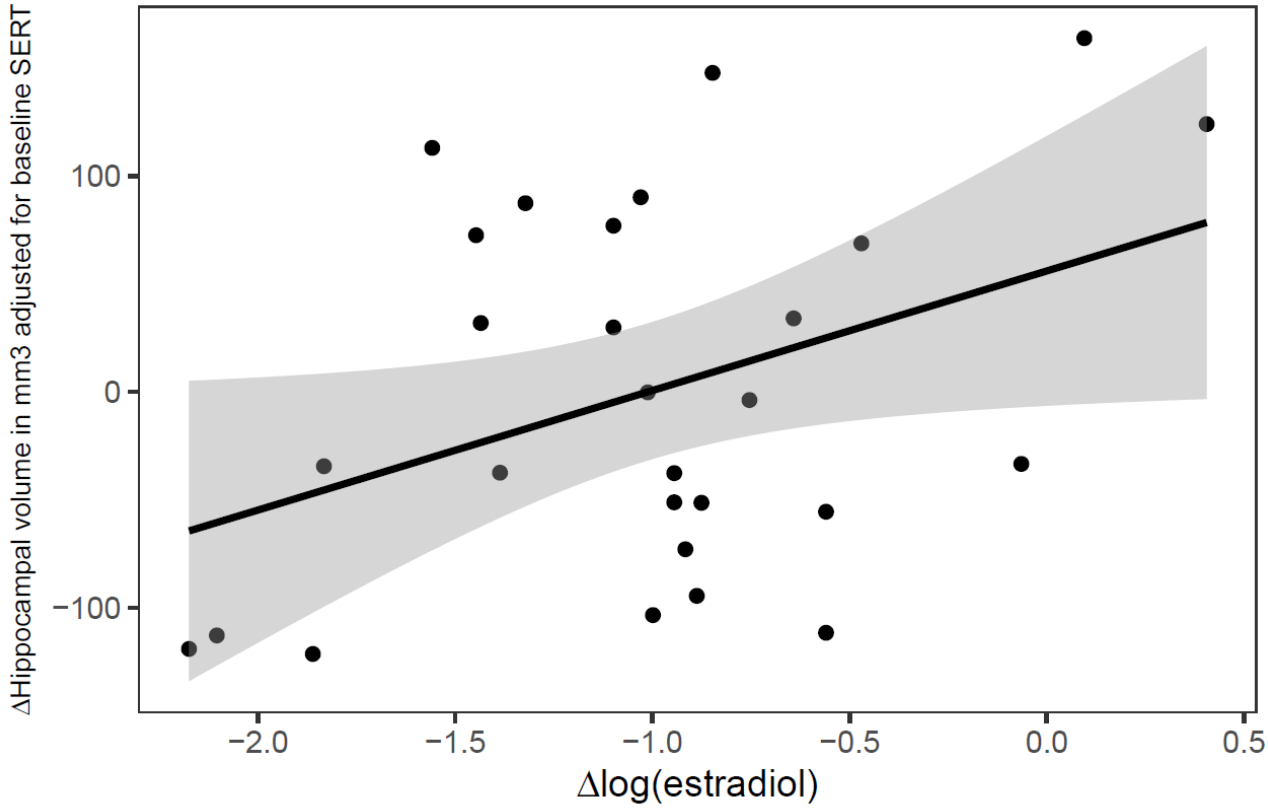


Figure 5. Association between estradiol and hippocampal volume in the GnRHa group. *GnRHa induced change in estradiol concentration was positively associated with change in hippocampal volume (partial r2 = 0.04, p = 0.04), in a model adjusted for SERT binding at baseline (residuals are used to visualize hippocampal volume controlled for baseline SERT binding).*



Highlights

- Placebo-controlled pharmaco-MRI sex-hormone manipulation study in healthy women
- Changes in estradiol and depressive symptoms were mapped across intervention
- Hippocampal volume changes and brain serotonin transporter availability were mapped

- Abrupt estradiol withdrawal reduced hippocampal volume in a non-depressogenic way
- Serotonergic markers contributed to hippocampal volume changes

Title: The role of central serotonergic markers and estradiol changes in perinatal mental health

Running title: Perinatal mental health: estradiol and serotonin

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Data availability statement

Data published in this manuscript are available through the Center for Integrated Molecular Brain Imaging database. The data are not publicly available due to privacy or ethical restrictions.

Abstract

Objective: Women have an increased risk for mental distress and depressive symptoms in relation to pregnancy and birth. The serotonin transporter (SERT) is involved in the emergence of depressive symptoms postpartum and during other sex-hormone transitions. It may be associated with cerebrospinal fluid (CSF) levels of the main serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA). In 100 healthy pregnant women planned to deliver by caesarean section (C-section), we evaluated 5-HIAA and estradiol contributions to mental distress five weeks postpartum.

Methods: Eighty-two women completed the study. CSF collected at C-section was analyzed for 5-HIAA, with high performance liquid chromatography. Serum estradiol concentrations were quantified by liquid chromatography tandem mass spectrometry before C-section and postpartum. Postpartum mental distress was evaluated with the Edinburgh Postnatal Depression Scale (EPDS). Associations between EPDS, 5-HIAA, and ΔEstradiol were evaluated in linear regression models adjusted for age, parity and SERT genotype.

Results: Higher levels of postpartum mental distress symptoms were, independently, positively associated with high antepartum CSF 5-HIAA levels with borderline significance ($p = 0.06$) and negatively associated with a large decrease in estradiol concentrations ($p = 0.007$).

Conclusion: In a cohort of healthy pregnant women, postpartum mental distress was higher in women with high antepartum CSF 5-HIAA and lower in women with a large perinatal estradiol decrease. We speculate that high antepartum 5-HIAA is a proxy of SERT levels, that carry over to the postpartum period and convey susceptibility to mental distress. Further, in healthy women the postpartum return to lower estradiol concentrations may promote mental well-being.

Key words: pregnancy; postpartum; mental health; serotonin; estradiol

Significant Outcomes

- A large decrease in estradiol from late in pregnancy to week five postpartum is associated with fewer postpartum mental well-being in healthy women.
- High cerebrospinal fluid levels of the main serotonin metabolite, 5-HIAA, in pregnancy is associated with more postpartum mental distress symptoms in healthy women (borderline significance).

Limitations

- Low level of clinically relevant symptoms limits generalizability to clinical cohorts
- Missing data reduce statistical power
- Longitudinal changes quantified for estradiol, but serotonergic markers and mental distress symptoms were both quantified cross-sectionally

Introduction

Peripartum mental distress symptoms, such as anxiety, sleep disturbances or “postpartum blues”, are common (about 40%, 40% and 50%, respectively) and predispose to manifest depressive episodes (1–6). About 10-15% of new mothers develop a depressive episode with onset during pregnancy or up to 4 weeks postpartum, known as perinatal depression (PND; 7–9). Intriguingly, the risk for a severe depression is particularly high within the first 4-8 weeks postpartum (10), which extend the DSM-V diagnostic criteria. This transition from pregnancy to early postpartum is characterized by dramatic sex-steroid fluctuations, especially for the main estrogen during the reproductive years estradiol (E2). Estradiol levels increase steadily to very high levels during pregnancy, but are reduced to hypogonadal levels within days after delivery and, in breastfeeding women, remain low for months (11,12). However, most studies have not been able to link absolute levels of estrogens directly to PND or mental distress, although some studies point towards an underlying sensitivity to estrogens at a genomic level (13–22). Intriguingly, E2 potently affects key features of the serotonin (5-HT) signaling system and induce expression of the main regulator of synaptic 5-HT: the serotonin transporter (SERT; 23–28). Some studies (29,30), but not all (31), suggest that the risk for postpartum depression is increased in women with high-expressing SERT genotypes in a gene dose-dependent manner, i.e., women who are homozygous for the long allele of the serotonin-transporter-linked promotor region (5-HTTLPR), have a higher risk than heterozygotes or short allele homozygotes. Evidence from a pharmacological sex-hormone manipulation risk model for depression indicate that a large net decrease in E2 may trigger depressive symptoms in *interaction* with higher brain SERT availability (32). In humans in vivo SERT imaging in pregnancy is not possible due to exposure to radiation, however some evidence can be provided from proxy markers of SERT induction in cerebrospinal fluid (CSF) and from studies in the immediate postpartum. CSF studies in humans and rodents, suggest that levels of the main serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) in CSF may serve as a proxy for SERT expression, as long-term reductions in SERT are associated with decreased levels of 5-HIAA in CSF (33–40). Also, monoamine oxidase type A (MAO-A) metabolizes the monoaminergic neurotransmitters, including 5-HT, and responds to E2 (41). Thus, 5-HIAA may also serve as a marker for MAO-A activity late in pregnancy. Intriguingly, human studies have shown that MAO-A may be important in postpartum mood regulation (42,43). Currently, it remains unclear how

pregnancy and the related increase in estrogens affects 5-HIAA concentrations in CSF and if 5-HIAA contribute to perinatal mental distress (44,45).

Thus, we do not know if the dramatic changes in E2 across the perinatal period contribute to mental distress in interaction with 5-HTTLPR, at least in some women, through a transiently compromised serotonin signaling. Under the assumption that induction of SERT increases 5-HIAA, we hypothesized that high 5-HIAA antepartum (late pregnancy) and a large decrease in E2 would be associated with more mental distress symptoms in postpartum women and that 5-HIAA and E2 would interact in inducing mental distress. Further, that the association between change in E2 and mental distress would be more pronounced in women homozygous for the high-expressing 5-HTTLPR variant.

Aims of the Study

In a longitudinal study of healthy pregnant women, we assessed if antepartum levels of 5-HIAA in CSF, 5-HTTLPR status and perinatal change in E2 contributed to mental distress or subclinical depressive symptoms five weeks postpartum.

Materials and methods

Participants

For the purpose of obtaining CSF for antepartum neurotransmitter quantification, only women planned to deliver by caesarean section (C-section) were eligible for the study. Thus, 100 healthy pregnant women in gestational week 38-42 and planned to deliver by C-section were recruited from obstetric departments at Copenhagen University Hospital, Denmark (Rigshospitalet and Herlev Hospital), for a study approved by the local ethics committee in the Capital Region of Denmark (protocol H-18029563). All participants gave written informed consent.

Inclusion criteria: age 18-40 years, planned C-section due to fetal breech position, previous C-section, previous myomectomy, obstructing fibroid, previous rupture of the anal sphincter, and uncomplicated placenta previa. Exclusion criteria: Previous or current severe somatic or psychiatric illness, pregestational Body Mass Index (BMI) below 18 or above 35, severe postpartum hemorrhage, infant with severe illness, use of medications that affect the central nervous system, substance abuse, non-fluent in Danish, severe learning disabilities, and impaired vision or hearing. Screening included questionnaires on mental well-being, medical history and

blood tests. The majority of the participants were Caucasians, but three were of Asian descent. Out of the included participants, eight had a psychiatric history of mild anxiety or depressive symptoms, including three cases of previous postpartum depression. Further two had previously experienced intrauterine fetal death or neonatal death. Two women had a possible thromboembolic episode in pregnancy and received subcutaneous injections of heparin, two women had a history of migraine, two had congenital urogenital anomalies, and one had a thyroid disease in remission. One third of the participants were included during the Severe Acute Respiratory Syndrome Coronavirus-2 pandemic of 2020 (COVID-19).

Out of the 100 women, four gave birth vaginally before the planned C-section and were thus excluded from the rest of the study. For the remaining 96, 14 did not complete follow-up due to: infants with severe illness (n = 3), personal reasons (n = 2), and lost contact with the research team (n = 9). Out of the 82 who completed, 19 had missing data at follow-up due to: COVID-19 related cancellations of blood samples (5), enrollment under an older protocol version (1), and non-compliance with questionnaires (13). Quantification of CSF markers failed for two participants and genotyping for one participant for technical reasons. Thus, complete data were available for 60. An overview of the study design can be found in Figure 1.

Questionnaires and interviews

Our main outcome was postpartum subclinical depressive symptoms evaluated with the Danish validated version of the Edinburgh Postnatal Depression Scale (EPDS; range: [0-30], cut-off for depression: >11; 46). For antepartum screening of participants and to evaluate longitudinal mood, sleep and anxiety symptoms, we used the Major Depressive Inventory (MDI; range: [0-50]; 47), the state subscale of the State Trait Anxiety Inventory (STAI; range: [20-80]; 48), and Pittsburg Sleep Quality Index (PSQI; range: [0-21]; 49). Participants completed MDI, STAI and PSQI shortly before the C-section (median: 1 day, range [0; 15], one C-section was postponed two weeks), and five weeks postpartum (median: 35 days, range: [16; 64], one participant came in late due to severe depression). EPDS was administered at week five postpartum (median: 35 days, range: [16; 64]). At week five postpartum, we used the Mini-International Neuropsychiatric Interview (M.I.N.I.) to rule out any undiagnosed severe psychiatric disorders (50).

Estrogen analyses

Serum samples for estrogen analyses were collected before C-section (median: 0 days, range: [0; 2]) and repeated five weeks postpartum (median: 35 days, range: [15; 66]). Serum samples were transferred to -20 °C immediately after centrifugation and collection. Concentrations of estrone (E1), E2 and estriol (E3) were measured simultaneously in serum samples by liquid chromatography tandem mass spectrometry (LC-MS/MS) with prior liquid–liquid extraction, as described in (51). In short, estrogens were purified from 200 µL thawed serum sample by liquid–liquid extraction using heptane/ethyl acetate followed by analysis on a Dionex UltiMate 3000 UHPLC system with integrated Transcend TLX TurboFlow sample preparation system, coupled with triple quadrupole mass spectrometer (TSQ Quantiva) from Thermo Scientific controlled by Aria MX 2.2 and Xcalibur 4.0 software. The TurboFlow- LC-MS/MS system was equipped with a loading Cyclone-P TurboFlow column followed by an analytical Kinetex® Phenyl-Hexyl column, for further sample extraction and chromatographic separation of the estrogens. The tandem mass spectrometry system was equipped with a heated electrospray ionization source (HESI) running in negative mode. The total duration time was 5.50 minutes.

Cerebrospinal fluid for neurotransmitter analyses

Cerebrospinal fluid (CSF) was collected as a part of the anesthetic procedures for the C-section. Briefly, anesthesiologists collected 0.5-1 ml of CSF during spinal anesthesia, which was immediately transferred to dry ice and subsequently stored at -80 °C.

CSF concentrations of 5-hydroxyindoleacetic (5-HIAA) , serotonin (5-HT), norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), and homovanillic acid (HVA) were assayed by high-performance liquid chromatography, as detailed in (52). Briefly, 10 µL of the samples was injected onto a Prodigy C18 column (100 x 2 mm I.D., 3-µm particle size, YMC Europe, Schermbeck, Germany) at flow rate of 0.15 mL/min. The mobile phase consisted of 55 mM sodium acetate, 1 mM octanesulfonic acid, 0.1 mM Na2EDTA and 7% Acetonitrile, adjusted to pH 3.7 with 0.1 M acetic acid, and was degassed using an on-line degasser. For the electrochemical detection, we used an amperometric detector (Antec Decade from Antec, Leiden, The Netherlands) with a glassy carbon electrode set at 0.7 V, with an Ag/AgCl as reference electrode. The output was recorded on

a computer program system CSW (Data Apex, Prague, The Czech Republic), which was used to calculate the peak areas.

5-HTTLPR genotyping

Analysis was performed on whole blood samples drawn prior to C-section and stored at −20 °C. Genotyping was done in line with previous studies, with some modifications (53). DNA was extracted from blood with a Chemagic DNA Blood 4k Kit H24 and a Chemagic 360-D instrument (PerkinElmer, Waltham, Massachusetts) according to the manufacturer's guidelines. 5-HTTLPR (SLC6A4; rs774676466) genotyping was performed using PCR amplification with the forward primer 5'-TAATGTCCCTACTGCAGCCC-3' and reverse primer 5'-GGGACTGAGCTGGACAACC-3'. PCR was performed in a total volume of 15 µL containing DNase/RNase-free distilled water, 100 ng DNA, 0.6 µmol/L of each primer (TAG Copenhagen, Denmark), 30 µmol/L dNTP (Qiagen, Hilden, Germany), commercial buffer and Taq DNA-polymerase (VWR, Radnor, Pennsylvania), and 12% (v/v) sucrose (Sigma-Aldrich®, Merck KGaA, Darmstadt, Germany). The PCR temperature cycling conditions were as follows: initial denaturation for 70 min at 98°C, followed by 40 cycles: denaturation at 96°C for 30 sec, primer annealing at 68°C for 30 sec, and primer extension at 72°C for 30 sec. The last cycle was followed by a final extension step for 7 min at 72°C. The PCR product was loaded on a 2% agarose gel in 1xTBE, and the fragments were separated by gel electrophoresis at 100V for 30 min. Gels were stained with SYBRTM Safe DNA Gel Stain (Thermo Fisher Scientific, Waltham, Massachusetts) and visualized with a ChemiDoc MP imaging system (Bio-Rad, Hercules, California). The genotypes were identified as LL 493 bp, LS 450bp and 493bp, and SS 450bp. For statistical analyses, we dichotomized the participants into long allele homozygotes (LL) or carriers of the short allele (S-carrier).

Statistical analyses

Prior to analyses, E2 was log-transformed (base = 2) to ensure normally distributed data. The ante- to postpartum change in E2 (ΔE2) concentration was derived as postpartum minus antepartum log-transformed concentrations.

To test our hypotheses, we used multiple linear regression models, all conducted in R (http://cran.r-project.org/). Since EPDS only is validated for postpartum use in Danish, we did not

collect antepartum EPDS and thus could not control postpartum scores for antepartum scores. We maintained this structure for other measures of mental distress, as antepartum scores may have been biased by the high stress situation prior to the C-section. Only one woman developed significant depressive symptoms, thus we were not able to describe the trajectory for severely depressed cases and we excluded her data from postpartum statistical analyses.

Because of missing data, we evaluated the main effects in models where *either* 5-HIAA *or* ΔE2 were included. The models were adjusted for known risk factors for perinatal depression, i.e., age, parity, 5-HTTLPR genotype (9,29,54–57). Log-likelihood-ratio test indicated that parity and 5-HTTLPR were relevant covariates, but not age or number of days between birth and postpartum follow-up. We kept age in the model, due to an uneven age distribution between primiparous women and women who were parous at inclusion. Further we evaluated if 5-HTTLPR genotype interacted with ΔE2 in the association with mental distress. Additionally, we evaluated if age and parity interacted with either ΔE2 or 5-HIAA. Interaction effects between 5-HIAA and ΔE2 were evaluated in complete cases only. We also report the contributions of 5-HIAA and ΔE2 mutually adjusted, in complete case data. Due to missing data, we additionally applied an inverse probability of censoring weights approach to the main model, see supplementary materials.

We considered the option that some of the associations for 5-HIAA might be established already in pregnancy and carried over to the postpartum period, thus we also evaluated antepartum 5-HIAA associations with available antepartum distress scores. Further, we evaluated if postpartum E2 mapped better on to EPDS than ΔE2.

Linear mixed-effects models with an unstructured covariance matrix were used to determine if mental distress varied across the whole perinatal period. Supplementary analyses conducted to evaluate potential bias can be found in supplementary materials and include baseline characteristics of drop-outs; antepartum associations between E2, 5-HIAA, 5-HT; contributions from other estrogens; differences between primiparous and multiparous women; and if variations in the timing of follow-up was important for postpartum E2. Our main hypothesis concerned the 5-HIAA, but alternative models for other monoaminergic neurotransmitters or their metabolites can also be found in supplementary materials.

The p-values are not adjusted for multiple comparisons. $p < 0.05$ was considered significant.

Results

Demographics and antepartum characteristics

Sample characteristics prior to delivery for the 96 women that underwent C-section are described in Table 1, note that at follow-up the number of participants with available data varied. Forty-four women underwent C-section due to fetal breech position; 39 due to previous C-section; 2 due to placenta previa; 7 due to previous rupture of anal sphincter; and 4 due to uterine fibroid or previous myomectomy. Mean age was 33.8 years. Scores of postpartum depressive symptoms were clearly within the normal spectrum (46,58). As expected, anxiety and sleep disturbances were, on average, close to or above suggested cut-offs (STAI: 34-40; PSQI: 5; Buysse et al., 1989; Tendais et al., 2014).

Mental distress and associations with 5-HIAA, E2, 5-HTTLPR, age and parity

ΔE2 was positively associated with EPDS at week five postpartum, i.e., a small decrease was associated with more symptoms ($\beta_{\Delta E2 =}$ 0.73, 95% CI: [0.21; 1.25], p = 0.007; Figure 2, panel A), in a model not adjusted for 5-HIAA. Similar results were found for postpartum E2, i.e., higher postpartum E2 was associated with more distress ($\beta_{E2\ postpartum:}$ 0.81, 95% CI: [0.26; 1.37], p = 0.005). In the same model, ΔE2 interacted with parity, such that a *large* decrease in E2 was associated with lower EPDS score in multiparous women, compared to primiparous women, who had no association between EPDS and ΔE2 ($\beta_{\Delta E2\text{-by-parity}}$ = 1.46, 95% CI:[-2.58; -0.33], p = 0.01). We found no significant interactions between ΔE2 and 5-HTTLR ($\beta_{\Delta E2\text{-by-5-HTTLPR}}$ = -0.81, 95% CI: [-2.12; 0.51], p = 0.22) or age ($\beta_{\Delta E2\text{-by-age}}$ = -0.02, 95% CI: [-0.17; 0.14], p = 0.84).

5-HIAA was positively associated with depressive symptoms with borderline significance ($\beta_{5\text{-HIAA}}$ = 0.002, 95% CI: [-7×10^{-5}; 0.004], p = 0.06; Figure 2, panel B), in a model not controlled for ΔE2. In this model, 5-HIAA did not interact with age ($\beta_{5\text{-HIAA-by-age}}$ = 0.0003, 95% CI: [-0.0003; 0.0008], p = 0.36) or parity ($\beta_{5\text{-HIAA-by-parity}}$ = -0.002, 95% CI: [-0.006; 0.002], p = 0.32).

When both ΔE2 and 5-HIAA were included in the model, i.e., when we used only complete case data (n=59), the effects sizes were similar however less significant ($\beta_{\Delta E2}$ = 0.63, 95% CI: [0.09; 1.16], p = 0.02; $\beta_{5\text{-HIAA}}$ = 0.002, 95% CI: [-0.0004; 0.004], p = 0.12). 5-HIAA and ΔE2 did not interact with each other in their association with postpartum mental distress ($\beta_{5\text{-HIAA-by-}\ \Delta E2}$ = -0.0002, 95% CI: [-0.001; 0.001], p = 0.79). Adjusted for both 5-HIAA and ΔE2, primiparous women had

significantly more symptoms on EPDS relative to multiparous women (β_{parity} = 1.66, [0.03; 3.29], p = 0.05), but neither age (β_{age} = 0.03, [-0.18; 0.25], p = 0.76) nor 5-HTTLPR were significantly associated with EPDS ($\beta_{5\text{-}HTTLPR}$ = 1.44, [-0.41; 3.29], p = 0.12). Postpartum mental distress evaluated with MDI, STAI and PSQI is detailed in Table 2 and supported that 5-HIAA and ΔE2 were associated with subclinical depressive symptoms and anxiety might, but that sleep disturbances might be more related to ΔE2. Further, 5-HIAA may have been positively associated with anxiety in pregnancy (STAI; $\beta_{5\text{-}HIAA}$: 0.006, 95% CI: [0.001; 0.01], p = 0.02), but not mood (MDI; $\beta_{5\text{-}HIAA}$: 0.001, 95% CI: [-0.002; 0.01], p = 0.36) or sleep (PSQI; $\beta_{5\text{-}HIAA}$: 0.0004, 95% CI: [-0.002; 0.002], p = 0.69). Linear mixed-effects models showed that, on average, MDI score decreased significantly across the antepartum to postpartum transition (estimate: -2.09, 95% CI: [-3.33; -0.84], p = 0.001). STAI scores followed a pattern similar to MDI (estimate: -5.6, 95% CI:[-7.36; -3.85], p <0.001). PSQI score did not change significantly from ante- to postpartum (estimate: 0.17, 95% CI:[-0.55; 0.88], p = 0.65).

Alternative models with other monoaminergic neurotransmitters and their metabolites (serotonin, norepinephrine, and 3-methoxy-4-hydroxyphenylglycol homovanillic acid) showed no statistically significant associations with EPDS, see supplementary materials. Results for the estrogens estrone (E1) and estriol (E3) were similar to those found for E2 and can be found in supplementary materials.

Discussion

In a longitudinal cohort study, we evaluated serotonergic and estrogenic contributions to maternal mental distress across the ante- to postpartum transition. High antepartum 5-HIAA was associated with the emergence of subclinical depressive symptoms five weeks postpartum, with borderline significance, while a large decrease in E2 from late in pregnancy to postpartum was associated with less depressive symptoms postpartum. The negative association between a large decrease in E2, between ante- and postpartum, and mental distress symptoms was more pronounced in multiparous women. Contrary to what we hypothesized, we observed no significant interaction between E2 and 5-HIAA or 5-HTTLPR.

Serotonergic CSF markers and perinatal mood

As hypothesized, 5-HIAA late in pregnancy was positively associated with the emergence of subclinical depressive symptoms and anxiety symptoms postpartum, although only on a borderline significant level (for EPDS). Antepartum, 5-HIAA was only significantly associated with anxiety symptoms. This suggests an underlying vulnerability in the serotonin system, which may induce some symptoms in pregnancy, that can be more fully developed into broader subclinical symptoms of depression by the ante- to postpartum transition. Thus, anxiety in pregnancy may be a marker for serotonergic changes that increase susceptibility to postpartum distress. In line with this, other studies have also found that anxiety in pregnancy increase the risk for postpartum depression (3,60). As detailed in supplementary materials, this underlying vulnerability was not likely to depend on antepartum E2 or 5-HTTLPR, as neither mapped on to 5-HIAA. Contrary to our expectations, 5-HTTLPR played no role in postpartum mood. This does not align with evidence from studies that report an increased risk for postpartum depression in women with high-expressing variants of 5-HTTLPR (29,30). However, these studies were conducted in clinical cohorts. Therefore, in our healthy cohort associations between 5-HTTLPR and postpartum mental distress may not be present or are too small to detect. This is supported by the model selection process, which indicated that inclusion of 5-HTTLPR improved model fit. Direct quantification of brain SERT also points towards an association between high SERT and mental distress in response to short-term sex-hormone fluctuations (32). Under the assumption that 5-HIAA is elevated as a consequence of pregnancy-induced SERT expression, that would be in line with our results (24,28,33–36,61). That is, increased SERT levels in pregnancy, observable as a high 5-HIAA in CSF, may carry over to the postpartum period and trigger mental distress postpartum. Perhaps this reflects that, in some women, SERT does not adapt (or down-regulate) appropriately in response to the high E2 levels late in pregnancy. This may be similar to the reduced flexibility in SERT regulation observed in individuals who develop a depression in response to seasonal changes (62). On the other hand, previous studies reported the same or higher 5-HIAA CSF concentrations in healthy pregnant women, compared to non-pregnant women, suggesting that high E2 in pregnancy not normally leads to reductions in 5-HIAA (44,45). Notably, neither study reports postpartum health, nor do they provide longitudinal data. Further, our sample is larger than

previous studies and we find a lower mean 5-HIAA and a wider range in concentrations, suggesting a greater variability than these studies capture.

5-HIAA is also regulated by MAO-A deamination of 5-HT, which E2 may inhibit (41). Human studies suggests that brain MAO-A distribution is higher early postpartum, where sex-steroid levels are low, and normalize in healthy postpartum women, but not in women with postpartum mood symptoms (43,63). However, as detailed in supplementary materials, antepartum E2 potentially mapped on to norepinephrine, but not 5-HIAA or 5-HT, suggesting that 5-HIAA levels not mainly were driven by E2 induction of MAO-A. Similarly, we show in supplementary materials that none of the other monoamines are associated with EPDS score, suggesting that neither MAO-A or -B are responsible for the association between E2 and 5-HIAA.

Thus, we speculate that high 5-HIAA mainly reflects pregnancy induced high SERT levels, which may carry over from antepartum to the early postpartum period and convey susceptibility to mental distress at least up to five weeks postpartum. The increased 5-HIAA may reflect a failure to adapt SERT levels in response to the high hormone concentrations late in pregnancy. Early effects of increased 5-HIAA leading to anxiety symptoms may be established already late in pregnancy and develop further across the ante-to postpartum transition.

Estradiol (E2)and perinatal mental distress

As expected, change in E2 across the ante- to postpartum transition was associated with postpartum mental distress, however the direction was opposite of what we hypothesized, i.e., a large change was associated with less postpartum distress. Thus, our results contrast findings from our sex-hormone manipulation model (32). The apparent disparities may be due to differences in the length and magnitude of the E2 concentration changes, as noted by others (28,64). For instance, hormone replacement during perimenopause has beneficial effects on hippocampal volume and depressive mood, but not when administered well beyond menopause (65–68). Similarly, high endogenous estrogens increase hippocampal volume during the menstrual cycle, while the long-term estrogen exposure during pregnancy is associated with a decrease in hippocampal volume (69,70). Thus, the long and high amplitude stimulation in pregnancy may promote different mechanisms than a brief hormone manipulation. Supplementary analyses indicated that the association between estradiol change and mental distress, mainly was driven by

postpartum E2 concentrations. Thus, healthy women seem to respond beneficially to a sudden downregulation, while women who develop a depression postpartum may not. This strongly support that estrogen sensitivity may be a risk marker for postpartum depression as indicated by recent genomic studies (16,18,71,72). This may explain the difference between the current study and those who report an that lower estradiol levels are associated with depression (15,73). At the same time, it indicates that the rapid E2 changes may be important for healthy postpartum adaptations. In line with this, antepartum E2 and working memory is positively associated in non-depressed women, while depressed women do not benefit in terms of cognitive performance from the high E2 in pregnancy, suggesting that the same mechanisms that provoke depressive symptoms, may provide an advantage in healthy women (74). Our results differ from the number of studies that have found no association between postpartum mood symptoms and absolute E2 levels or changes(14,17,18,20). However, there are multiple methodological differences that may explain this, such as sample size, degree of clinically relevant symptoms, absolute E2 levels versus changes and sensitivity of the quantification method. In the current study, we quantified E2 with high sensitivity methods that are able to accurately estimate the very low and very high E2 concentrations postpartum and in pregnancy (51). Thus, we may have captured associations previously missed by others. Of note, Klier et al. (17) found higher early postpartum estrogen levels in healthy pregnant women who developed a depression postpartum. An older study also reported that higher postpartum E2 might map on to mental distress (21). This is in accordance with our results where a high postpartum E2/smaller ante- to postpartum decrease in E2 was associated with more mental distress. This association was strong in multiparous women, independent of age, but not in primiparous women. Thus, previous pregnancy and birth may be an advantage for subsequent pregnancies, perhaps through altered hormone sensitivity or changes in brain structure (69,71). As detailed in supplementary materials, there were no absolute differences between multiparous or primiparous women to explain this, but parity-associated differences in E2 dynamics or steroid metabolism may have played a role. However, primiparity was also an independent risk factor for postpartum mental distress, in line with in other studies (9,54,60,75). Thus, primiparous women may simply be more sensitive to estradiol changes, for instance because they lack maternal experience and the first birth is a more life-changing event.

We speculate that the highly hormone stimulated state late pregnancy promotes perinatal mental wellbeing in most women, but that such protective mechanisms may be disrupted in women, who develop postpartum mental distress or depression. These protective effects seem to be enhanced by previous maternal experience.

Mental distress in the cohort

Only few women developed symptoms, compared to what we would expect from population studies (7). We observed a pattern reported by other studies, where depressive symptoms, in particular anxiety, are more prevalent late in pregnancy, compared to the postpartum period (17,76,77). To some extent, short term distress due to worry about the C-section may explain this, as emphasized by the rapid decline in anxiety symptoms postpartum (78). Sleep disturbances were stable across the whole perinatal period.

Methodological considerations

The most important limitation of this study is the absence of clinical levels of depressive symptoms, which may limit the reproducibility to women at risk for PND. This may be a consequence of the study design, which inherently has some selection bias towards robust individuals: C-section on low-stress indications, inclusion of a high proportion of multiparous women, exclusion of women with current diagnosed psychiatric illness and high average socioeconomic status at our inclusion sites. Participants reported increased focus on their own symptoms as a consequence of participation, but also a greater sense of safety. How this may have affected mental distress in the participants is unclear. Although approximately one third of the data were collected during the Severe Acute Respiratory Syndrome Coronavirus-2 pandemic of 2020, we did not observe an increase in distress scores or drop-out in this period (not reported). This is probably because women who consented to the study during in this period were more robust. However, we also included women with a higher risk of PND due to previous perinatal and non-perinatal depression, anxiety and adverse life-events (3,79–81). We do not know if the 14 women left the study before follow-up may have developed a depression, but as shown in supplementary materials, they suffered more from sleep disturbances in pregnancy, which is a known risk factor for depression (20,82). However, by studying these phenomena in healthy

women, we may have unmasked associations that would have been obscured by other risk factors for PND. Perhaps that is why we were able to detect a very discrete association in women with a limited range of distress scores. We were, however, limited by missing data, which, according to supplementary analyses, may have resulted in a lower statical significance for the 5-HIAA association with mental distress. Further, we have no information on the longitudinal changes in 5-HIAA and EPDS, for ethical and practical reasons.

Conclusion

In a cohort of healthy pregnant women, mental distress symptoms five week postpartum were higher in women with high antepartum levels of 5-HIAA in CSF and lower in women who experienced a large perinatal decrease in E2. The associations for 5-HIAA and E2 were independent. 5-HIAA may be associated with pregnancy induced SERT levels that carry over to the early postpartum period and convey susceptibility to mental distress. Postpartum return to lower hormone levels seem to protect mental wellbeing in healthy women.

References

1. Beck CT. Predictors of postpartum depression: An update. Nurs Res 2001;**50**:275–285.
2. Emamian F, Khazaie H, Okun ML, Tahmasian M, Sepehry AA. Link between insomnia and perinatal depressive symptoms: A meta-analysis. J Sleep Res 2019;**28**:e12858.
3. Robertson E, Grace S, Wallington T, Stewart DE. Antenatal risk factors for postpartum depression: A synthesis of recent literature. Gen Hosp Psychiatry 2004;**26**:289–295.
4. Grant KA, McMahon C, Austin MP. Maternal anxiety during the transition to parenthood: A prospective study. J Affect Disord 2008;**108**:101–111.
5. Richter D, Krämer MD, Tang NKY, Montgomery-Downs HE, Lemola S. Long-term effects of pregnancy and childbirth on sleep satisfaction and duration of first-time and experienced mothers and fathers. Sleep 2019;**42**. doi:10.1093/sleep/zsz015
6. Salari N, Darvishi N, Khaledi-Paveh B et al. A systematic review and meta-analysis of prevalence of insomnia in the third trimester of pregnancy. BMC Pregnancy Childbirth 2021;**21**:1–8.
7. Meltzer-Brody S, Boschloo L, Jones I, Sullivan PF, Penninx BW. The EPDS-Lifetime: Assessment of lifetime prevalence and risk factors for perinatal depression in a large cohort of depressed women. Arch Womens Ment Health 2013;**16**:465–473.
8. American Psychiatric Association. DSM-5 Task Force. *Diagnostic and statistical manual of mental disorders : DSM-5.* American Psychiatric Association, 2013
9. Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal depression: A systematic review of prevalence and incidence. Obstet Gynecol 2005;**106**:1071–1083.
10. Putnam KT, Wilcox M, Robertson-Blackmore E et al. Clinical phenotypes of perinatal depression and time of symptom onset: analysis of data from an international consortium. The Lancet Psychiatry 2017;**4**:477–485.
11. McNeilly AS. Breastfeeding and the suppression of fertility. Food Nutr Bull 1996;**17**:340–345.
12. Kuijper EAM, Ket JCF, Caanen MR, Lambalk CB. Reproductive hormone concentrations in

pregnancy and neonates: A systematic review. Reprod. Biomed. Online. 2013;**27**:33–63.

13. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of gonadal steroids in women with a history of postpartum depression. Am J Psychiatry 2000;**157**:924–930.

14. Chatzicharalampous C, Rizos D, Pliatsika P et al. Reproductive hormones and postpartum mood disturbances in Greek women. Gynecol Endocrinol 2011;**27**:543–550.

15. Fan F, Zou Y, Ma A, Yue Y, Mao W, Ma X. Hormonal changes and somatopsychologic manifestations in the first trimester of pregnancy and post partum. Int J Gynecol Obstet 2009;**105**:46–49.

16. Guintivano J, Arad M, Gould TD, Payne JL, Kaminsky ZA. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. Mol Psychiatry 2014;**19**:560–567.

17. Klier CM, Muzik M, Dervic K et al. The role of estrogen and progesterone in depression after birth. J Psychiatr Res 2007;**41**:273–279.

18. Mehta D, Newport DJ, Frishman G et al. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Psychol Med 2014;**44**:2309–2322.

19. Osborne LM, Gispen F, Sanyal A, Yenokyan G, Meilman S, Payne JL. Lower allopregnanolone during pregnancy predicts postpartum depression: An exploratory study. Psychoneuroendocrinology 2017;**79**:116–121.

20. Okun ML, Luther J, Prather AA, Perel JM, Wisniewski S, Wisner KL. Changes in sleep quality, but not hormones predict time to postpartum depression recurrence. J Affect Disord 2011;**130**:378–384.

21. Heidrich A, Schleyer M, Spingler H et al. Postpartum blues: Relationship between not-protein bound steroid hormones in plasma and postpartum mood changes. J Affect Disord 1994;**30**:93–98.

22. O'Hara MW, Schlechte JA, Lewis DA, Wright EJ. Prospective Study of Postpartum Blues: Biologic and Psychosocial Factors. Arch Gen Psychiatry 1991;**48**:801–806.

23. Sumner BEH, Grant KE, Rosie R, Hegele-Hartung C, Fritzemeier KH, Fink G. Raloxifene blocks estradiol induction of the serotonin transporter and 5-hydroxytryptamine2A receptor in female rat brain. Neurosci Lett 2007;**417**:95–99.

24. Lu NZ, Eshleman AJ, Janowsky A, Bethea CL. Ovarian steroid regulation of serotonin reuptake

transporter (SERT) binding, distribution, and function in female macaques. Mol Psychiatry 2003;**8**:353–360.

25. Suda S, Segi-Nishida E, Newton SS, Duman RS. A Postpartum Model in Rat: Behavioral and Gene Expression Changes Induced by Ovarian Steroid Deprivation. Biol Psychiatry 2008;**64**:311–319.

26. Bethea CL, Lu NZ, Gundlah C, Streicher JM. Diverse actions of ovarian steroids in the serotonin neural system. Front. Neuroendocrinol. 2002;**23**:41–100.

27. Borrow AP, Cameron NM. Estrogenic mediation of serotonergic and neurotrophic systems: Implications for female mood disorders. Prog Neuro-Psychopharmacology Biol Psychiatry 2014;**54**:13–25.

28. Deecher D, Andree TH, Sloan D, Schechter LE. From menarche to menopause: Exploring the underlying biology of depression in women experiencing hormonal changes. Psychoneuroendocrinology. 2008;**33**:3–17.

29. Sanjuán J, Martin-Santos R, Garcia-Esteve L et al. Mood changes after delivery: Role of the serotonin transporter gene. Br J Psychiatry 2008;**193**:383–388.

30. Tavares Pinheiro R, Monteiro Da Cunha Coelho F, Azevedo Da Silva R et al. Association of a serotonin transporter gene polymorphism (5-HTTLPR) and stressful life events with postpartum depressive symptoms: A population-based study. J Psychosom Obstet Gynecol 2013;**34**:29–33.

31. Hu J, Zhou B, Li Y et al. The interaction between estradiol change and the serotonin transporter gene (5-HTTLPR) polymorphism is associated with postpartum depressive symptoms. Psychiatr Genet 2019;**29**:97–102.

32. Frokjaer VG, Pinborg A, Holst KK et al. Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: A positron emission tomography study. Biol Psychiatry 2015;**78**:534–543.

33. Carpenter LL, Anderson GM, Siniscalchi JM, Chappell PB, Price LH. Acute changes in cerebrospinal fluid 5-HIAA following oral paroxetine challenge in healthy humans. Neuropsychopharmacology 2003;**28**:339–347.

34. Y S, ME B, JG C. Correlated reductions in cerebrospinal fluid 5-HIAA and MHPG concentrations after treatment with selective serotonin reuptake inhibitors. J Clin

Psychopharmacol 1997;**17**:11–14.

35. De Bellis MD, Geracioti TD, Altemus M, Kling MA. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. Biol Psychiatry 1993;**33**:636–641.

36. Potter WZ, Scheinin M, Golden RN et al. Selective Antidepressants and Cerebrospinal Fluid: Lack of Specificity on Norepinephrine and Serotonin Metabolites. Arch Gen Psychiatry 1985;**42**:1171–1177.

37. Williams RB, Marchuk DA, Gadde KM et al. Serotonin-related gene polymorphisms and central nervous system serotonin function. Neuropsychopharmacology 2003;**28**:533–541.

38. Mårtensson B, Wägner A, Beck O, Brodin K, Montero D, Åsberg M. Effects of clomipramine treatment on cerebrospinal fluid monoamine metabolites and platelet H-imipramine binding and serotonin uptake and concentration in major depressive disorder. Acta Psychiatr Scand 1991;**83**:125–133.

39. Bartolomucci A, Carola V, Pascucci T et al. Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. DMM Dis Model Mech 2010;**3**:459–470.

40. Licht CL, Knudsen GM, Sharp T. Effects of the 5-HT4 receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. Neurosci Lett 2010;**476**:58–61.

41. Lokuge S, Frey BN, Foster JA, Soares CN, Steiner M. Depression in women: Windows of vulnerability and new insights into the link between estrogen and serotonin. J Clin Psychiatry 2011;**72**:e1563–e1569.

42. Sacher J, Wilson AA, Houle S et al. Elevated brain monoamine oxidase A binding in the early postpartum period. Arch Gen Psychiatry 2010;**67**:468–474.

43. Sacher J, Rekkas PV, Wilson AA et al. Relationship of monoamine oxidase-A distribution volume to postpartum depression and postpartum crying. Neuropsychopharmacology 2015;**40**:429–435.

44. Altemus M, Fong J, Yang R, Damast S, Luine V, Ferguson D. Changes in cerebrospinal fluid neurochemistry during pregnancy. Biol Psychiatry 2004;**56**:386–392.

45. Spielman FJ, Mueller RA, Corke BC. Cerebrospinal fluid concentration of 5-hydroxyindoleacetic acid in pregnancy. Anesthesiology 1985;**62**:193–195.

46. Smith-Nielsen J, Matthey S, Lange T, Væver MS. Validation of the Edinburgh Postnatal

Depression Scale against both DSM-5 and ICD-10 diagnostic criteria for depression. BMC Psychiatry 2018;**18**:393.

47. Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. J Affect Disord 2001;**66**:159–164.

48. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Manual for the State-Trait Anxietry, Inventory. Palo Alto, CA Consult Psychol Press 1983.

49. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;**28**:193–213.

50. Sheehan D V., Lecrubier Y, Sheehan KH 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 1998;**59**:22–33.

51. Frederiksen H, Johannsen TH, Andersen SE et al. Sex-specific Estrogen Levels and Reference Intervals from Infancy to Late Adulthood Determined by LC-MS/MS. J Clin Endocrinol Metab 2020;**105**:754–768.

52. Weikop P, Kehr J, Scheel-Krüger J. Reciprocal effects of combined administration of serotonin, noradrenaline and dopamine reuptake inhibitors on serotonin and dopamine levels in the rat prefrontal cortex: the role of 5-HT 1A receptors. J Psychopharmacol 2007;**21**:795–804.

53. Kalbitzer J, Erritzoe D, Holst KK et al. Seasonal Changes in Brain Serotonin Transporter Binding in Short Serotonin Transporter Linked Polymorphic Region-Allele Carriers but Not in Long-Allele Homozygotes. Biol Psychiatry 2010;**67**:1033–1039.

54. Munk-Olsen T, Jones I, Laursen TM. Birth order and postpartum psychiatric disorders. Bipolar Disord 2014;**16**:300–307.

55. Muraca GM, Joseph KS. The Association Between Maternal Age and Depression. J Obstet Gynaecol Canada 2014;**36**:803–810.

56. García-Blanco A, Monferrer A, Grimaldos J et al. A preliminary study to assess the impact of maternal age on stress-related variables in healthy nulliparous women. Psychoneuroendocrinology 2017;**78**:97–104.

57. Aasheim V, Waldenström U, Hjelmstedt A, Rasmussen S, Pettersson H, Schytt E. Associations

between advanced maternal age and psychological distress in primiparous women, from early pregnancy to 18 months postpartum. BJOG An Int J Obstet Gynaecol 2012;**119**:1108–1116.

58. Bech P, Timmerby N, Martiny K, Lunde M, Soendergaard S. Psychometric evaluation of the Major Depression Inventory (MDI) as depression severity scale using the LEAD (Longitudinal Expert Assessment of All Data) as index of validity. BMC Psychiatry 2015;**15**. doi:10.1186/s12888-015-0529-3

59. Tendais I, Costa R, Conde A, Figueiredo B. Screening for depression and anxiety disorders from pregnancy to postpartum with the EPDS and STAI. Span J Psychol 2014;**17**:E7.

60. Blackmore ER, Gustafsson H, Gilchrist M, Wyman C, O'Connor TG. Pregnancy-related anxiety: Evidence of distinct clinical significance from a prospective longitudinal study. J Affect Disord 2016;**197**:251–258.

61. Bethea CL, Reddy AP, Tokuyama Y, Henderson JA, Lima FB. Protective actions of ovarian hormones in the serotonin system of macaques. Front Neuroendocrinol 2009;**30**:212–238.

62. Mc Mahon B, Andersen SB, Madsen MK et al. Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder. Brain 2016;**139**:1605–1614.

63. Sacher J, Wilson AA, Houle S et al. Elevated brain monoamine oxidase A binding in the early postpartum period. Arch Gen Psychiatry 2010;**67**:468–474.

64. Rehbein E, Hornung J, Sundström Poromaa I, Derntl B. Shaping of the Female Human Brain by Sex Hormones: A Review. Neuroendocrinology 2021;**111**:183–206.

65. Albert K, Hiscox J, Boyd B, Dumas J, Taylor W, Newhouse P. Estrogen enhances hippocampal gray-matter volume in young and older postmenopausal women: a prospective dose-response study. Neurobiol Aging 2017;**56**:1–6.

66. Gordon JL, Rubinow DR, Eisenlohr-Moul TA, Xia K, Schmidt PJ, Girdler SS. Efficacy of transdermal estradiol and micronized progesterone in the prevention of depressive symptoms in the menopause transition: A randomized clinical trial. JAMA Psychiatry 2018;**75**:149–157.

67. Lord C, Buss C, Lupien SJ, Pruessner JC. Hippocampal volumes are larger in postmenopausal women using estrogen therapy compared to past users, never users and men: A possible

window of opportunity effect. Neurobiol Aging 2008;**29**:95–101.

68. Resnick SM, Espeland MA, Jaramillo SA et al. Postmenopausal hormone therapy and regional brain volumes: The WHIMS-MRI Study. Neurology 2009;**72**:135–142.

69. Hoekzema E, Barba-Müller E, Pozzobon C et al. Pregnancy leads to long-lasting changes in human brain structure. Nat Neurosci 2017;**20**:287–296.

70. Pletzer B, Harris TA, Scheuringer A, Hidalgo-Lopez E. The cycling brain: menstrual cycle related fluctuations in hippocampal and fronto-striatal activation and connectivity during cognitive tasks. Neuropsychopharmacology 2019;**44**:1867–1875.

71. Mehta D, Rex-Haffner M, Sondergaard HB, Pinborg A, Binder EB, Frokjaer VG. Evidence for oestrogen sensitivity in perinatal depression: Pharmacological sex hormone manipulation study. Br J Psychiatry 2019;**215**:519–527.

72. Osborne L, Clive M, Kimmel M et al. Replication of Epigenetic Postpartum Depression Biomarkers and Variation with Hormone Levels. Neuropsychopharmacology 2016;**41**:1648–1658.

73. O'Hara MW, Schlechte JA, Lewis DA, Varner MW. Controlled Prospective Study of Postpartum Mood Disorders: Psychological, Environmental, and Hormonal Variables. J Abnorm Psychol 1991;**100**:63–73.

74. Hampson E, Phillips SD, Duff-Canning SJ et al. Working memory in pregnant women: Relation to estrogen and antepartum depression. Horm Behav 2015;**74**:218–227.

75. Atar Gürel S, Gürel H. The evaluation of determinants of early postpartum low mood: The importance of parity and inter-pregnancy interval. Eur J Obstet Gynecol Reprod Biol 2000;**91**:21–24.

76. Heron J, O'Connor TG, Evans J, Golding J, Glover V. The course of anxiety and depression through pregnancy and the postpartum in a community sample. J Affect Disord 2004;**80**:65–73.

77. Evans J, Melotti R, Heron J et al. The timing of maternal depressive symptoms and child cognitive development: A longitudinal study. J Child Psychol Psychiatry Allied Discip 2012;**53**:632–640.

78. Matthey S, Ross-Hamid C. Repeat testing on the Edinburgh Depression Scale and the HADS-A in pregnancy: Differentiating between transient and enduring distress. J Affect Disord

2012;**141**:213–221.

79. GUINTIVANO J, SULLIVAN PF, STUEBE AM et al. Adverse life events, psychiatric history, and biological predictors of postpartum depression in an ethnically diverse sample of postpartum women. Psychol Med 2018;**48**:1190–1200.

80. WISNER KL, PEREL JM, PEINDL KS, HANUSA BH. Timing of depression recurrence in the first year after birth. J Affect Disord 2004;**78**:249–252.

81. MELTZER-BRODY S, MAEGBAEK ML, MEDLAND SE, MILLER WC, SULLIVAN P, MUNK-OLSEN T. Obstetrical, pregnancy and socio-economic predictors for new-onset severe postpartum psychiatric disorders in primiparous women. In: Psychological Medicine. 2017, 1427–1441.

82. MAGHAMI M, SHARIATPANAHI SP, HABIBI D et al. Sleep disorders during pregnancy and postpartum depression: A systematic review and meta-analysis. Int J Dev Neurosci 2021;**81**:469–478.

Tables

Table 1. Antepartum characteristics, neurotransmitter concentrations, peripartum mental distress and peripartum estradiol concentrations.

	Antepartum (n = 96)	**Week five postpartum (n = 82)**
Demographics		
Parity at inclusion (0 / ≥ 1 child)	38/ 57 [a]	-
Age Mean (SD) / Median (range)	34 (3) / 34 (23; 41)	-
5-HTTLPR genotype LL vs. S-carrier	28 / 67 [b]	-
Sex of child (Boy/Girl)	52/44	-
E2 and 5-HIAA		
Estradiol (E2) ***in pmol/L*** Mean (SD)	79387 (26630)	81 (152)[g]
Change in E2 ***(pmol/L)*** Mean (SD)	-	-78585 (27949)
5-HIAA ***in CSF in fmol/10µL*** Mean (SD)	1164.86 (366.71)[c]	-
Mental distress		
EPDS Mean (SD)	-	4.06 (4.22)[h]
MDI Mean (SD)	8.12 (5.25)	6.45 (6.07)[h]
STAI [e] Mean (SD)	32.51 (9.02)	27.17 (8.85) [h]

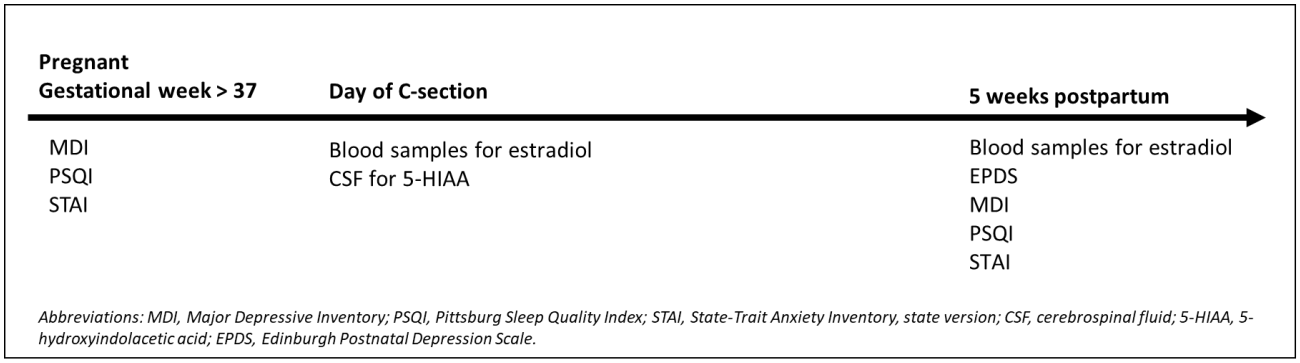
PSQI [f] Mean (SD)	6.74 (3.2)	7.07 (3) [h]
[a] n = 95, parity not logged for one dropout; [b] n = 95, analysis failed for one woman; [c] n = 94, analysis failed for two women; [e] n = 92; [f] n = 86; [g] n = 76; [h] n = 69		

Table 2. 5-HIAA and ΔE2 associations with postpartum anxiety, sleep disturbances and mood, mutually adjusted and controlled for 5-HTTLPR, parity and age.

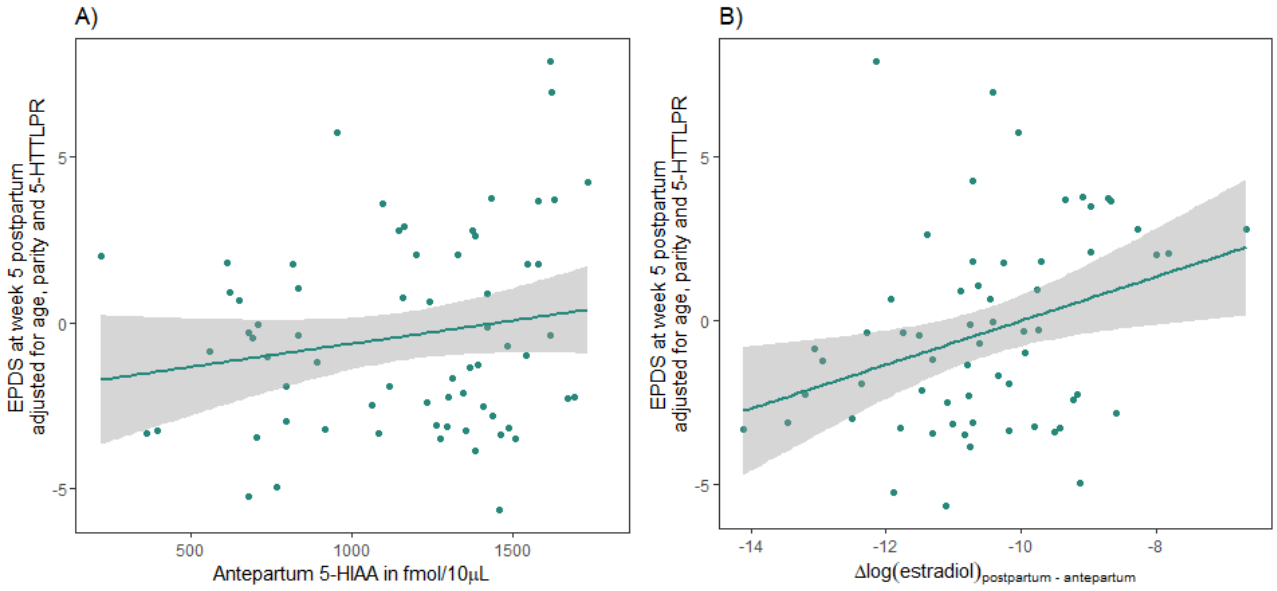
	MDI		***STAI***		***PSQI***	
Covariate	*β (95% CI)*	*p* [a]	*β (95% CI)*	*p* [a]	*β (95% CI)*	*p* [a]
5-HIAA	0.003 (0.0006; 0.006)	0.02	0.004 (8×10^{-5}; 0.008)	0.05	0.002 (-0.002; 0.002)	0.81
ΔE2	0.77 (0.07; 1.46)	0.03	0.21 (0.08; 2.34)	0.04	0.71 (0.27; 1.14)	0.002
[a] Unadjusted for multiple comparisons						

Figure legends

Figure 1. Overview of study design.



Legend to Figure 2. A) Association between 5-HIAA and EPDS residuals after adjusting for age, parity and 5-HTTLPR. Linear regression line has 95% CI. B) Association between ΔE2 and EPDS residuals after adjusting for age, parity and 5-HTTLPR.
Linear regression line has 95% CI depicted in gray shade. Note that residuals and not EPDS scores are on the y-axis


A)
EPDS at week 5 postpartum
adjusted for age, parity and 5-HTTLPR
5
0
-5
500
1000
1500
Antepartum 5-HIAA in fmol/10µL
B)
EPDS at week 5 postpartum
adjusted for age, parity and 5-HTTLPR
5
0
-5
-14
-12
-10
-8
Δlog(estradiol)postpartum - antepartum

Supplementary materials

CSF analyses

Antepartum CSF

Mean antepartum concentrations of CSF markers can be found in Supplementary table 1.

Supplementary Table 1. Mean concentrations of CSF markers antepartum	
	CSF marker concentrations in fmol/10µL
5-HIAA Mean (SD)	1164.86 (366.71)
5-HT Mean (SD)	69.91 (21.99)
NE Mean (SD)	104.41 (46.2)
HVA Mean (SD)	1025.5 (498.45)
MOPEG Mean (SD)	474.92 (216.88)

Antepartum associations between E2, serotonergic CSF markers and age

Antepartum associations between CSF markers and E2, controlled for age and 5-HTTLPR can be found in Table 2. We observed a trend level interaction between 5-HTTLPR and antepartum E2 on 5-HT, such that low-expressing variants had higher 5-HT in CSF ($\beta_{E2*5\text{-}HTTLPR}$ = 16.61, 95% CI: [-4.23; 37.46], p = 0.12). No such effect was found for 5-HIAA ($\beta_{E2*5\text{-}HTTLPR}$ = 208.67, p = 0.24). 5-HTTLPR had no interaction effects with age on 5-HIAA or 5-HT (p-values > 0.88).

Supplementary table 2. Associations between antepartum E2, age and the CSF markers: 5-HIAA , serotonin (5-HT), norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MOPEG) and homovanilic acid (HVA)

CSF marker	**β_{E2}**	***p***	**β_{Age}**	***p***	**$\beta_{5\text{-}HTTLPR}$**	***p***
5-HIAA	16.81	0.84	1.1	0.92	-85,3	0.32

5-HT	1.62	0.74	0.47	0.5	2.1	0.68
NE	-16.55	0.11	-1.27	0.31	-	-
MOPEG	66.69	0.16	-4.94	0.4	-	-
HVA	-108.34	0.33	-20.67	0.13	-	-

Main models for 5-HT

Supplementary table 3: 5-HT associations with postpartum EPDS score, adjusted for ΔE2, age, parity and 5-HTTLPR		
	β	*p*
5-HT	-0.01	0.47
5-HT-by-ΔE2	-0.01	0.24
5-HT-by-parity	0.03	0.33
5-HT-by-age	-0.004	0.34
5-HT-by-5-HTTLPR	-0.01	0.82

Alternative models for the remaining CSF markers

Supplementary Table 4. The association between week five EPDS and non-serotonergic CSF markers. Markers were log-transformed due to non-normality. Both HVA and NE may have been associated with EPDS, but not MHPH, which is the metabolite of NE. Thus, MAO-A induction does not seem to be the underlying reason.

CSF-M	**$\beta_{\text{CSF-M controlled for age, parity and 5-HTTLPR}}$**	P-unadjusted
Log(MHPG)	0.19	0.74
Log(HVA)	0.77	0.07
Log(NE)	0.81	0.13

Drop-outs

We evaluated if there were any differences in baseline characteristics between women who completed follow-up versus women who left the study. For this, we used Wald-type t-tests and Fisher's exact tests.

Women who did not complete the study, had more disturbed antepartum sleep on a borderline significant level (mean difference in PSQI: 2.77, 95% CI: [-0.24; 5.78], p = 0.07). There were no significant differences between those who left the study before week five and those that remained in terms of parity (OR = 1.08, 95% CI: [0.28; 4.57], p = 1), sex of child (OR = 1.15, 95% CI: [0.317; 4.41], p = 1), age (mean difference: -0.91, 95% CI: [-3.7; 1.89], p = 0.5), 5-HTTLPR (OR = 2, 95% CI: [0.51; 7.45], p = 0.34), antepartum E2 concentrations (mean difference: -1360, 95% CI [-14766.27; 12044.27], p = 0.84), 5-HIAA (mean difference: -128.08, 95% CI: [-373.43; 117.26], p = 0.29), antepartum MDI score (mean difference = 0.87, 95% CI [-2.85; 4.59], p = 0.63) or antepartum STAI score (mean difference: 1.167, 95% CI: [-4.68; 7.018], p= 0.68).

Primiparous women versus multiparous women

First-time mothers were younger than women who were parous at inclusion (mean difference: 2.4 years, 95% CI: [0.79;4.05], p = 0.004). There was no significant difference between the groups in terms of the sex of the child (OR = 0.9, 95% CI: [0.36; 2.22], p = 0.84) and 5-HTTLPR (OR = 0.64, 95% CI: [0.24; 1.75], p = 0.36). Mean antepartum estradiol concentrations did not differ significantly (multi: 79805; primi: 79701, p = 1). There were no significant difference in absolute postpartum estradiol concentrations (multi 94.21; primi 65.55, p = 0.36), adjustment for days postpartum for follow-up did not change this (parity: -31.16, p = 0.4;
days:2.53, p = 0.24

Estrogens

Alternative models with E1 and E3 instead of E2:

At week 5 change in E1 (**β** = 0.84, p = 0.03), but not E3 (**β** = -0.28, p = 0.7) was associated with EPDS.

Correlations between estrogens ante- and postpartum

Antepartum estriol (E3) was highly and significantly correlated with E2 (Pearson's correlation coefficient E3: 0.75, p < 0.001). Antepartum estrone (E1) was less correlated with E2, but still at a significant level (Pearson's correlation coefficient E1: 0.55, p < 0.001).
Postpartum E3 did not correlate with E2, but E1 did (Pearson's correlation coefficient E3: -0.04, p = 0.71; Pearson's correlation coefficient E1: 0.95, p < 0.001). Change in E1, but not E3, correlated with change in E2 (Pearson's correlation coefficient E1: 0.59, p < 0.001; Pearson's correlation coefficient E3: 0.12, p = 0.3).
Mean values and mean change in E1 and E2 can be found in Supplementary Table 3.

Supplementary Table 2.

	Antepartum (n = 96)	**Week five postpartum (n = 76)**
Serum estrogens in pmol/L		
Estrone (E1) Mean (SD)	28898 (18380)	103 (91)
Estriol (E3) Mean (SD)	49008 (18111)	12 (0.3)
Change in E1 Mean (SD)	-	-27612 (16412)
Change in E3 Mean (SD)	-	-49522 (18957)

Estradiol postpartum and number of days since birth

Not all women came in for follow-up exactly at the same number of days postpartum. Thus, we evaluated if the number of days between the two E2 measurements were associated with the

magnitude of the decrease. However, we also considered if this was moderated by factors known to influence E2 dynamics in pregnancy, i.e., age, parity and sex of the child (Schock et al., 2016; Toriola et al., 2011).

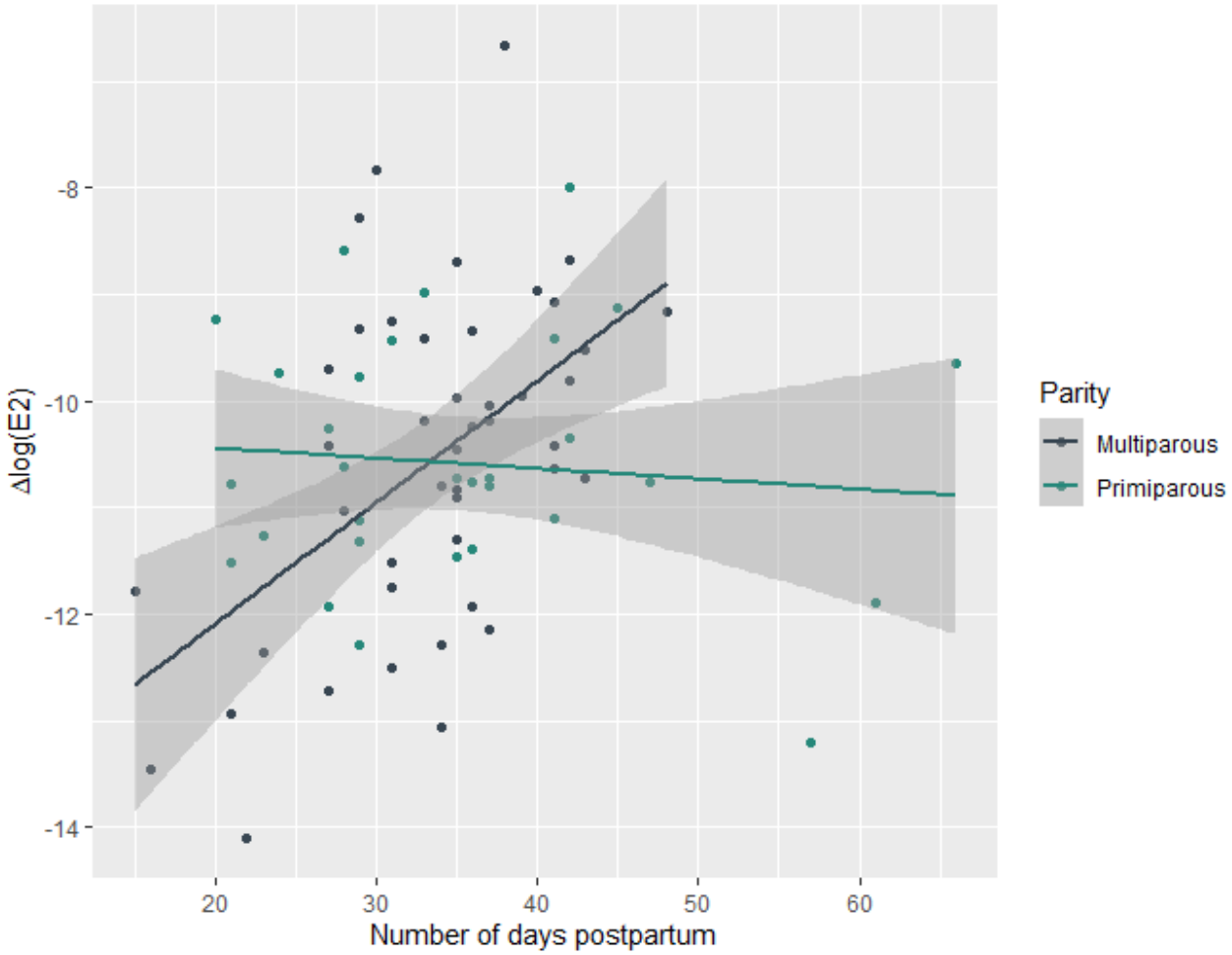
Among the multiparous women, the magnitude of E2 decrease from antepartum values was associated with time postpartum such that the earlier the larger the decrease. No such association could be observed for primiparous women. This effect was significant both when looking at absolute postpartum E2 concentration (β = -0.09, 95% CI: [-0.16; -0.02], p = 0.008) and ΔE2 (-0.12; 95%CI: [-0.19; -0.05], p < 0.001), see Supplementary Figure 1.

The direction of these effects was also the same for E1 and E3, but was only statistically significant for E1 ($\beta_{\Delta E1}$: -0.07, p = 0.005; $\beta_{E1\ postpartum}$: -0.04; p = 0.02). No such effects were found for age (p = 0.25), although as expected the trajectory of the older age group followed that of multiparous women. A similar interaction effect was found for sex of the child, although at a less significant level, such that women who gave birth to girls had decreased more in E2 early postpartum and less later postpartum, than those who had boys ($\beta_{girls\text{-}by\text{-}E2}$: 0.08, 95% CI: [4.06; 0.16], p = 0.05). Neither E1 or E3 had interaction effects with sex of child or age (all p > 0.46).

We do not know if this is a spurious effect, but we speculate that it may be due to higher steroid metabolism and/or less prolactin induced suppression of the sex-hormone axis in parous women(Alberto Zuppa et al., 1988; Bernstein et al., 1985). It is possible that the fast return to higher E2 levels in multiparas is a consequence of a steep decrease early postpartum. Longitudinal postpartum E2 data are required to explore this further, however others have reported differences in E2 levels between nulliparous and parous women (Bernstein et al., 1985; Schock et al., 2016; Toriola et al., 2011).

As we only measured E2 once postpartum, we do not know if the observed difference in dynamics is a spurious effect, or reflects faster E2 dynamics in multiparous women compared to primiparous women.

Supplementary Figure 1. Peripartum E2 dynamics. Week five samples were not all taken exactly 35 days after delivery but 15-64 days postpartum. The amount of time that elapsed between the C-section and the week five blood sample was associated with the magnitude of the perinatal decrease in E2, in a manner that depended on parity. For multiparous women, the more days that passed before the week five blood sample, the smaller the difference between postpartum and antepartum E2 concentrations.



Inverse probability censoring weighting – missing data

Inverse probability censoring weighting assigns allows for an estimation of associations, in cases where there is missing data for the outcome. Weights were based on mood symptoms one week postpartum (blues and MDI), as this may be associated with risk for depression later (Beck, 2001); PSQI antepartum, because dropouts had more sleep symptoms in pregnancy, and antepartum estradiol because we did not have follow-up estradiol for everybody. Inverse probability censoring weighting of the models with 5-HIAA, age, parity and 5-HTTLPR to some extend supported the results reported in the main paper. However, they indicated that the statistical significance for the association between 5-HIAA and EPDS may have been higher ($\beta_{5\text{-HIAA}}$ = 0.003, p= 0.03). Models with ΔE2 age, parity and 5-HTTLPR found similar results ($\beta_{\Delta E2}$ = 0.87, p = 0.007). In a model with ΔE2, 5-HIAA age, parity and 5-HTTLPR, parity seemed to be less significant, while 5-HTTLPR was more, age remained non-significant (parity: 1.55, p = 0.08; age: 0.07, p = 0.53; 5-HTTLPR: 2.1, p = 0.04).

References

Alberto Zuppa, A., Tornesello, A., Papacci, P., Tortorolo, G., Segni, G., Lafuenti, G., Moneta, E.,

Diodato, A., Sorcini, M., Carta, S., 1988. Relationship between maternal parity, basal prolactin levels and neonatal breast milk intake. Neonatology 53, 144–147. https://doi.org/10.1159/000242775

Beck, C.T., 2001. Predictors of postpartum depression: An update. Nurs. Res. 50, 275–285. https://doi.org/10.1097/00006199-200109000-00004

Bernstein, L., Pike, M.C., Ross, R.K., Judd, H.L., Brown, J.B., Henderson, B.E., 1985. Estrogen and sex-hormone-binding globulin levels in nulliparous and parous women. J. Natl. Cancer Inst. 74, 741–745.

Schock, H., Zeleniuch-Jacquotte, A., Lundin, E., Grankvist, K., Lakso, H.Å., Idahl, A., Lehtinen, M., Surcel, H.M., Fortner, R.T., 2016. Hormone concentrations throughout uncomplicated pregnancies: A longitudinal study. BMC Pregnancy Childbirth 16, 1–11. https://doi.org/10.1186/s12884-016-0937-5

Toriola, A.T., Vääräsmäki, M., Lehtinen, M., Zeleniuch-Jacquotte, A., Lundin, E., Rodgers, K.G., Lakso, H.A., Chen, T., Schock, H., Hallmans, G., Pukkala, E., Toniolo, P., Grankvist, K., Surcel, H.M., Lukanova, A., 2011. Determinants of maternal sex steroids during the first half of pregnancy. Obstet. Gynecol. 118, 1029–1036. https://doi.org/10.1097/AOG.0b013e3182342b7f

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Abstract

In the peripartum period, women commonly experience mental distress symptoms that may develop into depressive episodes. The serotonin 4 receptor (5-HT4R) seems to be sensitive to sex-hormones and may have antidepressant properties directly relevant in postpartum distress and depression, perhaps in a manner coupled to hippocampal plasticity. The aim of this study was to assess hippocampal volume and cerebral 5-HT4R in postpartum women versus healthy female controls and relate imaging parameters to the degree of mental distress symptoms and the peripartum decrease in estradiol (ΔE2).

We quantified 5-HT4R brain binding with positron emission tomography and hippocampal volume with magnetic resonance imaging in 31 postpartum women and 24 healthy female controls. Mental distress was assessed postpartum with the Hamilton 17-item depression scale. With linear regression, we evaluated age-adjusted group differences in hippocampal volume and 5-HT4R brain binding and postpartum associations between mental distress, 5-HT4R, ΔE2 and hippocampal volume.

None of the women developed a depression. Cerebral 5-HT4R and hippocampal volume was similar in postpartum women and controls. High postpartum hippocampal volume was positively associated with mental distress ($p=0.04$). A large ΔE2 in interaction with high 5-HT4R was associated with decreased postpartum hippocampal volume (neostriatum: $p=0.02$, hippocampus: $p=0.003$).

Conclusion: Our findings indicate that the brain adapts well to peripartum sex-hormone changes , in the absence of clinical depression. However, higher postpartum hippocampal volume may reflect maladaptive peripartum hippocampal plasticity and increased vulnerability to mental distress. Further, 5-HT4R in interaction with peripartum ΔE2 may be linked to postpartum hippocampal plasticity.

Introduction

Across pregnancy and the early postpartum, women commonly experience mild to moderate mental distress, including anxiety, sleep disturbances and “postpartum blues”, which may predispose to manifest depressive episodes [1–4]. Further, depressive episodes with debut in pregnancy or up to four weeks postpartum, known as perinatal depression (PND), occur in up to 10-15% of pregnant and postpartum women [5–7]. Notably, the risk for depressive episodes extends beyond week four postpartum and is particularly high in the first 4-8 weeks postpartum [8].

Intriguingly, this early postpartum period is also characterized by the most dramatic changes in hormone milieu, as the steady rise in especially estrogens during pregnancy is followed by an abrupt decrease after delivery, and a continued suppression of ovarian hormone production in breastfeeding women [9–11]. Evidence from a human risk model for depression, suggests that maladaptive changes to the estrogen estradiol (E2) may trigger depressive symptoms in a manner dependent on serotonin-transporter (SERT) availability in the brain [12]. Correspondingly, high-expressing SERT genotypes increase the risk for depression in relation to pregnancy and child birth as seen in clinical cohorts [13,14]. In a cohort of healthy pregnant women, we found that high cerebrospinal fluid levels of the main serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in late pregnancy characterized women that were more vulnerable to postpartum mental distress [15]. Increased 5-HIAA may be a result of SERT induction in pregnancy [16–21] and thus represent a proxy for SERT availability.

Thus, there are some indications that high SERT availability late in pregnancy may be part of a natural brain response that in most women only transiently affect their levels of mental distress, but at the same time, in the case of maladaptation, may pose a risk for perinatal depressive responses.

Intriguingly, genetic and pharmacological studies in both humans and rodents indicate that SERT availability affects the serotonin 4 receptor (5-HT4R)[22–24]. Rodent studies point towards anxiolytic, pro-cognitive and antidepressant properties of 5-HT4R, including prevention of anhedonia [25]. Both cognitive problems, anxiety and anhedonia are key features of PND [8,26,27]. Human studies are less clear, but unpublished data suggests that 5-HT4R may be reduced in patients with major depression[28]. Further, 5-HT4R may be sensitive to sex-hormone changes [29]. In the human central nervous system, 5-HT4R is particularly abundant in striatum, hippocampus and to a lesser degree

in neocortex, although the antidepressant-like effect seen in rodent models may be exerted, in part, through prefrontal regulation of neuronal firing in the dorsal raphe nuclei and thus overall serotonergic tone in the brain [22,25]. However, rodent data also indicate that 5-HT4R may be involved in regulation of hippocampal plasticity [30].

Intriguingly, animal models show that the perinatal period is characterized by high hippocampal plasticity, which may be key in pregnancy-induced mechanism that support healthy maternal behavior and to some extent regulated by estrogens [31,32]. Further, recent human studies show that hippocampal volume becomes reduced over the course of pregnancy and postpartum, as a part of a functional remodeling [33]. Since hippocampal volume reductions are characteristic for patients with major depression [34], postpartum depression may be associated with dysfunctional remodeling mechanisms. We have previously shown that flexibility in the serotonin system may play a role for hippocampal volume changes in response to pharmacologically induced sex-hormone changes (Borgsted et al., in revision). Thus, it is possible that changes in serotonin signaling play a role in peripartum hippocampal remodeling or (mal)adaptations to perinatal transition.

We do not know if hippocampal volume and/or 5-HT4R may be involved in the emergence of mental distress symptoms postpartum and if this maps on to previously reported associations between pre- to postpartum E2 changes, 5-HIAA, and postpartum mental distress.

In the current study, we evaluated the difference in hippocampal volume and 5-HT4R brain binding between healthy postpartum women and healthy female controls. Further, we evaluated the role of hippocampal volume and cerebral 5-HT4R binding in postpartum mental distress, taking potential contributions from 5-HIAA and change in E2 into account. Additionally, we evaluated if antepartum CSF 5-HIAA concentration was coupled to postpartum 5-HT4R brain binding. Finally, we evaluated if cerebral 5-HT4R, changes in circulating E2 concentrations, and hippocampal volume were associated.

We hypothesized that:

a) healthy postpartum women have a higher 5-HT4R brain binding than healthy controls, but a smaller hippocampal volume;

b) both 5-HT4R binding and hippocampal volume postpartum would be inversely related to postpartum mental distress, in a manner that depended on change in E2 concentrations

c) 5-HT4R binding postpartum was associated with antepartum 5-HIAA;

d) hippocampal volume was associated with 5-HT4R availability and change in E2 concentrations.

Methods

Participants

100 healthy women pregnant in week 38-42 planned to deliver by caesarean section (C-section) were recruited from the obstetric departments at Rigshospitalet and Herlev Hospital, both Copenhagen University Hospital, Denmark, for a study approved by the local ethics commitee in the Capital Region of Denmark (protocol H-18029563). A subgroup of these were recruited for a neuroimaging program approved under the same ethics protocol. The aim was to include 20-25 healthy women for 5-HT4R positron emission tomography (PET) scans with ^{11}C-SB207145 and 25-35 for structural magnetic resonance imaging (MR).

Thirty-one consented to structural MR scans, out of the 31, 23 also consented to ^{11}C-SB207145 PET scans. In the following we report our findings based on the imaging subgroup. All participants gave written informed consent.

Inclusion criteria were the same as for the main study: age 18-40 years, planned C-section due to fetal breech position, previous C-section, previous myomectomy, obstructing fibroid, previous rupture of the anal sphincter, and uncomplicated placenta previa. In addition to the exclusion criteria for the main study, contraindications for brain scans were added. Thus, the exclusion criteria were: severe somatic or psychiatric illness (previous or current), pregestational BMI <18 or >35, severe postpartum hemorrhage, severely ill infant , use of medications with central nervous system effects, alcohol or substance abuse, non-fluent in Danish, severe learning disabilities, impaired vision or hearing, contraindications for MR scans, exposure to radioactivity of >10 mSv within the last year. Screening included questionnaires on mental well-being, medical history and blood tests. We administered the Mini-International Neuropsychiatric Interview (M.I.N.I.) postpartum to rule out undiagnosed severe psychiatric disorders [35].

While our main objective was to associate neurobiological markers in postpartum women to mental distress, we considered it relevant to provide a context for such associations. Thus, neuroimaging data from healthy controls, recruited for other neuroimaging projects conducted

approximately in the same period and under the same conditions, were chosen from the CIMBI database [36]based on the following criteria: Female sex, age 18-40 years, no use of hormonal contraceptives, ^{11}C-SB207145 images and/or structural MR scans acquired on the same scanners that we used for postpartum women. 24 healthy controls fitted the criteria and had undergone ^{11}C-SB207145 on the same scanner as the postpartum women. Out of the 24 controls, 22 were scanned on the same MR scanner as the postpartum women. In relation to their participation in other projects the controls were screened thoroughly for mental and physical health issues, including substance abuse, use of drugs with effects on the central nervous system and BMI <18 or >35. The controls had not given birth recently.

Structural MR acquisition and hippocampal volume estimation

T1-weighted structural MR scans were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. Image acquisition parameters: inversion time = 900 ms, TE = 2.58 ms, TR = 1900ms, flip angle = 9°, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap.

For the purpose of hippocampal volume extraction, T1-weighted images underwent automatic subcortical segmentation and parcellation[37,38] with the standard cross-sectional pipeline in FreeSurfer 7.1[39] (http://surfer.nmr.mgh.harvard.edu/). Quality-control steps were in line with the ENIGMA protocols (http://enigma.usc.edu/). The volume of left and right hippocampus was extracted for statistical analysis. In all analyses, we used a mean of the left and right hippocampal volume. For the purpose of delineation of regions of interest in PET analyses, T1-weighted images were also segmented into white matter, grey matter and CSF in SPM8[40] (http://www.fil.ion.ucl.ac.uk/spm/).

5-HT4 receptor imaging with ^{11}C-SB207145 PET

For 5-HT4R imaging we used the radioligand ^{11}C-SB207145. Radioactive labelling with ^{11}C is particularly suitable for this type of scan, as it has a very short half-life of approximately 20 min. To further minimize radiation exposure, we aimed at a low injected radioactive dose (mean: 503.3 MBq, SD: 10.7). For the duration of the PET scan, the infant was left in the care of either a close relative or a member of the research team, to ensure that no infants were exposed to

radioactivity. Mother and child were reunited when 10 half-lives had passed (approximately 3.5 hours after injection of the tracer).

Acquisition of ^{11}C-SB207145 PET images and subsequent data analysis was otherwise conducted as previously described [23,41]. Briefly, after bolus injection of ^{11}C-SB207145 participants underwent a 120-minute dynamic PET scan on a high-resolution research tomography PET scanner (CTI/Siemens, Knoxville, TN, USA).

Motion corrected PET images were aligned and co-registered to the corresponding MR image before automated regional delineation in Pvelab [42]. Regional time-activity curves were extracted for kinetic modelling in PMOD (PMOD version 3.0, Zurich, Switzerland). We used the simplified reference tissue model with cerebellum as reference region, to quantify non-displaceable binding potentials (BPNDs) [43,44]. BPNDs from neostriatum, neocortex and hippocampus were extracted for statistical analysis.

Questionnaires and interviews:

Within the postpartum group, mental distress/subclinical levels of depressive symptoms were quantified with the Danish validated version of the Edinburgh Postnatal Depression Scale (EPDS) [45] and the Hamilton 17-item depression interview (HAMD-17) [46]. EPDS and HAMD-17 were administered at week one and five postpartum. At week five, HAMD-17 was completed on the PET scan day or, for the MR-only participants, on the MR scan day.

For postpartum versus control group characteristics, we used the Major Depressive Inventory (MDI)[47].

Estradiol (E2) analyses:

Blood samples for E2 analyses were collected before C-section (median: 0 days, range: [0; 2]), most often at the day of the procedure, and repeated five weeks postpartum in relation to brain scans (median: 35 days, range: [15; 66]). After spinning, serum was transferred to -20 °C. E2 concentrations were measured in serum samples by liquid chromatography tandem mass spectrometry (LC-MS/MS) with prior liquid–liquid extraction, as previously described [48]. In short, estrogens were purified from 200 µL thawed serum sample by liquid–liquid extraction using heptane/ethyl acetate. Thereafter the extracts were analyzed on an on-line TurboFlow-LC-MS/MS

system equipped with a heated electrospray ionization source (HESI) running in negative mode. The total duration time was 5.50 minutes.

Cerebrospinal fluid for neurotransmitter analyses

Cerebrospinal fluid (CSF) was collected as a part of the anesthetic procedures for the C-section. Briefly, anesthesiologists collected 0.5-1 ml of CSF through the spinal needle before inducing spinal anesthesia, which was immediately transferred to dry ice and subsequently stored at -80 °C. CSF concentrations of 5-hydroxyindoleacetic (5-HIAA) were assayed by high-performance liquid chromatography, as previously described [49]. Briefly, 10 µl of the sample was injected onto a Prodigy C18 column (100 x 2 mm I.D., 3-µm particle size, YMC Europe, Schermbeck, Germany) at flow rate of 0.15 mL/min. The mobile phase consisted of 55 mM sodium acetate, 1 mM octanesulfonic acid, 0.1 mM Na2EDTA and 7% Acetonitrile, adjusted to pH 3.7 with 0.1 M acetic acid, and was degassed using an on-line degasser. For the electrochemical detection, we used an amperometric detector (Antec Decade from Antec, Leiden, The Netherlands) with a glassy carbon electrode set at 0.7 V, with an Ag/AgCl as reference electrode. The output was recorded on a computer program system CSW (Data Apex, Prague, The Czech Republic), which was used to calculate the peak areas.

5-HTTLPR genotyping

Whole blood for DNA analyses was drawn prior to C-section and stored at −20 °C.. DNA was extracted from blood with a Chemagic 360-D (PerkinElmer, Waltham, Massachusetts). 5-HTTLPR (SLC6A4; rs774676466) genotyping was performed by PCR amplification with the forward primer 5'-TAATGTCCCTACTGCAGCCC-3' and reverse primer 5'-GGGACTGAGCTGGACAACC-3'. The fragments were then separated by gel electrophoresis. For statistical analyses, we dichotomized the participants into long allele homozygotes (LL) or carriers of the short allele (S-carrier).

Statistical analyses

All statistical analyses were performed in R v. 4.0.3 (http://cran.r-project.org/). Group differences in age and MDI were evaluated with Wald-type t-tests. Differences in distribution of 5-HTTLPR genotype were evaluated with Fisher's exact test. We used linear regression models adjusted for

age to determine if there were any statistically significant differences between healthy postpartum women and healthy controls in terms of 5-HT4R and hippocampal volume. Within the postpartum group, we used multiple linear regression models to determine if hippocampal volume or 5-HT4R brain binding was associated with EPDS and HAMD-17 at week five postpartum.. However, to capture the subclinical postpartum spectrum better we also included EPDS as outcome variable. Based on previous analyses in the larger cohort, from which these participants were recruited, ante- to postpartum ΔE2, antepartum 5-HIAA, age, parity and 5-HTTLPR genotype were considered potentially relevant covariates. Further, MR scans for hippocampal volume were not always conducted on the same postpartum day as HAMD-17 and EPDS were administered. The number of days between distress rating and MR scan were considered a potential covariate for the hippocampal volume analyses. However, to avoid overfitting of the model in a small sample size, we used the Akaike information criterion (AIC) for model selection . Accordingly, we constrained our model to include parity, ΔE2 and, for hippocampal volume, number of days between rating and MR as covariates. Notably, ΔE2 only improved the fit for EPDS models, but to ensure a consistent analysis framework for overlapping psychometrics i.e., HAMD-17 and EPDS, we also included it in HAMD-17 models.

Further, we used simple linear regression models to evaluate associations between 5-HT4R brain binding and 5-HIAA. Using multiple linear regression models, we both evaluated if hippocampal volume was associated with 5-HT4R brain binding and ΔE2, and if there was an interaction between 5-HT4R and ΔE2 on hippocampal volume. Based on previously published data on hippocampal volume[33,50] we included parity and age as covariates. Since MR imaging was often conducted on a different day than the PET scans and blood samples (the PET and blood samples were always acquired on the same day), the time difference was considered a potential covariate. However, it did not improve the model fit evaluated with AIC, nor did it change any association substantially. Thus, we did not include it in our model (see supplementary materials).

The reported p-values are not adjusted for multiple comparisons. $p < 0.05$ was considered significant.

Results

Descriptive statistics

Postpartum women were significantly older than healthy controls (mean difference: 9.45 years, 95% CI: [7.25; 11.65], $p < 0.001$). The distribution of 5-HTTLPR genotypes did not differ significantly between postpartum (8 LL and 23 S-carriers) and healthy controls (8 LL and 16 S-carriers; $p = 0.57$). Postpartum women did not differ significantly from healthy controls in terms of MDI (mean difference: 0.23, 95% CI: [-1.82; 2.29], $p = 0.82$). Detailed characteristics for the postpartum group can be found in **Table 1.** Postpartum women were MR scanned a mean of 30 days (range: [20; 43]) postpartum and PET scanned a mean of 29 days postpartum (range: [15; 42]). On average, 5 days passed between MR scans and blood samples/HAMD-17 (range: [-13; 16]) and 6 days passed between MR and EPDS (range: [-21; 16]). On average, 7 days passed between MR and PET scans (range:[-13;16]).

Postpartum women compared to healthy controls: 5-HT4R binding and hippocampal volume

Controlled for age, cerebral 5-HT4R binding was not significantly different between postpartum women and healthy controls in neostriatum (β_{group} = 0.13, 95% CI: [-0.35; 0.6], $p = 0.6$; β_{age} = -0.01, 95% CI: [-0.05; 0.03],$p = 0.63$; Figure 1), neocortex (β_{group} =0.003, 95% CI: [-0.08; 0.09], $p = 0.95$; β_{age} = -0.003, 95% CI: [-0.01; 0.004], $p = 0.42$) or hippocampus (β_{group} =0.1, 95% CI: [-0.09; 0.29], $p = 0.31$; β_{age} = 0.002, 95% CI: [-0.01; 0.02], $p = 0.77$). Not adjusted for age, group differences in 5-HT4R were also not significant (neostriatum, mean difference: 0.22, 95% CI: [-0.52; 0.09], $p = 0.17$; neocortex, mean difference: 0.03, 95% CI: [-0.09; 0.03], $p = 0.29$; hippocampus, mean difference: 0.08, 95% CI: [-0.2; 0.05], $p = 0.23$). Similarly, there was not a significant difference between postpartum women and healthy controls in terms of mean hippocampal volume, both adjusted for age (β_{group} = 154.86, 95% CI: [-122.07; 431.79], $p = 0.27$; β_{age} = 5.86, 95% CI: [-16.82; 28.54], $p = 0.61$; Figure 1) and not adjusted for age (mean difference: 100.42, 95% CI: [-77.96; 278.8], $p = 0.26$).

Mental distress, hippocampal volume and 5-HT4R binding in postpartum women

Controlled for parity and ΔE2, we observed no association between EPDS or HAMD-17 and 5-HT4R binding in neostriatum (HAMD-17: $\beta_{5\text{-HT4R}}$ = 0.52, 95% CI: [-1.32; 2.35], $p = 0.56$; EPDS: $\beta_{5\text{-HT4R}}$

=0.94, 95% [-3.25; 1.36], p = 0.40) or neocortex (HAMD-17: $\beta_{5\text{-HT4R}}$ =6.36, 95% CI: [-3.95; 16.67], p = 0.21; EPDS: $\beta_{5\text{-HT4R}}$ = -3.03, 95% CI: [-9.82; 15.89], p = 0.63). There was a positive association between EPDS, HAMD-17 and binding in hippocampus (HAMD-17: $\beta_{5\text{-HT4R}}$ =4.65, 95% CI: [0.88; 8.42], p = 0.02; EPDS: $\beta_{5\text{-HT4R}}$ = 4.86, 95% CI: [0.56; 9.15], p = 0.03), but this was primarily driven by one single observation with a relatively high score on HAMD-17 and EPDS. Without this observation, the association between hippocampal 5-HT4R and distress was not significant (HAMD-17: $\beta_{5\text{-HT4R}}$ = 1.87, 95% CI: [-2.49; 6.23], p = 0.38; EPDS: $\beta_{5\text{-HT4R}}$ = 2.95, 95% CI: [-2.85; 8.75], p = 0.27) and 5-HT4R in neocortex and neostriatum continued not to be associated with distress (all p > 0.4). We also evaluated if this association depended on a change in E2. There was no significant interaction between 5-HT4R binding in neocortex, neostriatum, and hippocampus, and a change in E2 on EPDS and HAMD-17 (p > 0.12), also when ignoring data from the person with high distress scores (all p > 0.15).

Hippocampal volume was associated with both EPDS and HAMD-17 (HAMD-17: $\beta_{\text{hippocampal volume}}$ = 0.002, 95% CI: [0.0001; 0.004], p = 0.04; EPDS: $\beta_{\text{hippocampal volume}}$ =0.003, 95% CI: [-0.0001; 0.005], p = 0.06, Figure 2) with borderline significance in a model controlled for the number of days that passed between rating and MR scan, parity, and ΔE2. Hippocampal volume did not interact with ΔE2 on EPDS or HAMD-17 (p > 0.43).

Postpartum 5-HT4R binding association with 5-HIAA

We observed no direct associations between 5-HT4R binding and antepartum 5-HIAA in neostriatum ($\beta_{5\text{-HIAA}}$ = -0.00006, 95% CI: [-0.0006; 0.0004], p = 0.81), neocortex ($\beta_{5\text{-HIAA}}$ = 0.00001,95% CI: [-0.0001; 0.0001], p = 0.75) or hippocampus (hippo: $\beta_{5\text{-HIAA}}$ = 0.0001, 95% CI: -0.0001; 0.0003], p = 0.28).

Association between hippocampal volume, 5-HT4R binding and estradiol in postpartum women

Within the postpartum group, we also evaluated the role of 5-HT4R and ΔE2 in hippocampal volume, in a model adjusted for age and parity. 5-HT4R binding in neostriatum ($\beta_{5\text{-HT4R}}$ = -11.74, 95% CI: [-322; 299], p = 0.94), neocortex ($\beta_{5\text{-HT4R}}$ = -343, 95% CI: [-2140; 1453], p = 0.69) and hippocampus ($\beta_{5\text{-HT4R}}$ = 123, 95% CI: [-640; 886], p = 0.74) were not associated with hippocampal

volume. Nor was ΔE2, regardless of the 5-HT4R region employed in the model (all $p > 0.41$). However, 5-HT4R binding in neostriatum and hippocampus interacted with ΔE2, such that higher binding and a small decrease in E2 promoted a higher hippocampal volume (neostriatum: $\beta_{5\text{-HT4R-by-}\Delta E2} = 258$, 95% CI: [50; 466], $p = 0.02$; hippocampus: $\beta_{5\text{-HT4R-by-}\Delta E2} = 905.54$, 95% CI: [358; 1453], $p = 0.003$, Figure 3). A similar, but less significant, interaction effect was found for neocortex ($\beta_{5\text{-HT4R-by-}\Delta E2} = 986$, 95% CI: [-85; 2057], $p = 0.07$). The associations remained similar when ignoring data from the person with high distress scores.

Discussion

In a combined PET and MR study, we mapped 5-HT4R brain binding and hippocampal volume in postpartum women, who displayed only minor mental distress symptoms postpartum. Contrary to what we expected, mean 5-HT4R brain binding and hippocampal volume postpartum was not significantly different from healthy controls. However, within the postpartum group, larger hippocampal volume, but not 5-HT4R brain binding, was associated with more subclinical depressive symptoms, i.e., higher HAMD-17 and EPDS scores, on a borderline significant level. Intriguingly, 5-HT4R in all regions, most pronounced in hippocampus, interacted with ante- to postpartum changes in E2, such that high 5-HT4R binding combined with a small pre- to postpartum change in E2, was associated with a larger hippocampal volume.

5-HT4R brain binding and perinatal transition

We were not able to confirm our hypothesis regarding a significant difference between healthy controls and postpartum women in terms of 5-HT4R binding. Multiple studies have reported perinatal changes in the serotonin system [51–53] and a number of studies point towards a regulatory role of E2 on several parts of the serotonin system [54,55]. This includes the 5-HT4R, which for example appears downregulated during oral contraceptive use, perhaps as a consequence of a blunted endogenous E2 dynamics [29]. However, it is possible that these hormonally driven serotonergic changes all counterbalance, as indicated by a stable 5-HT4R availability postpartum relative to non-postpartum, and maintained a stable 5-HT4R across pregnancy and postpartum.

Further, postpartum levels of 5-HT4R may be a result of rapid adjustment to the postpartum hormonal and neurobiological milieu, which may also serve to maintain good mental health. In support of a fast postpartum readjustment of 5-HT4R, antepartum cerebrospinal fluid markers of serotonergic turnover (5-HIAA) and postpartum serotonergic brain imaging markers (5-HT4R) were not significantly associated in the perinatal period. Although previous studies have reported an inverse relationship between sub-chronic serotonin levels and 5-HT4R, they found no acute effects on 5-HT4R[23]. In other words, such an association depends on stable conditions. However, the perinatal period is characterized by rapid changes in hormone milieu and remodeling of the brain architecture [33,56], which may result in a temporary uncoupling of such mechanisms. Thus, the serotonin system may have undergone multiple adaptations ante- to postpartum, which also may explain why measures of 5HIAA antepartum and 5-HT4R binding postpartum are not associated. Further, 5-HIAA is also regulated by many other factors, especially SERT availability[16,18,19,57,58], which may not be linked to 5-HT4R in the postpartum state.

Although animal studies indicate that 5-HT4R agonism may have anxiolytic, antidepressant and pro-cognitive properties [25], we observed no direct association between 5-HT4R and postpartum mental distress, except for in hippocampus. The association between mental distress and 5-HT4R in hippocampus was driven by a single observation with high mental distress scores; thus, we interpret this as a spurious finding. However, as discussed below 5-HT4R in interaction with E2 may have affected mood through hippocampal volume changes.

Taken together, postpartum women did not differ from healthy controls in terms of brain 5-HT4R availability and postpartum 5-HT4R did not map on to serotonergic markers collected in pregnancy. We do not know how pregnancy affects 5-HT4R or if 5-HIAA mapped on to 5-HT4R levels in pregnancy, but it is possible that 5-HT4R expression adapted rapidly to the postpartum state in healthy women as a part of a healthy return to the non-pregnant state in the brain.

Hippocampal volume

We observed no significant difference in terms of hippocampal volume between healthy controls and postpartum women. This was surprising given recent reports suggesting that pregnancy and birth is associated with a reversible reduction in hippocampal volume [33,59]. However, the current study is cross-sectional and we can therefore not determine if there were subtle within-subject

changes in hippocampal volume over the course of pregnancy, which remained within the limits of a normal hippocampal volume. Although our controls were mismatched with regards to age, it is not likely that age-matched controls would have had a higher hippocampal volume than younger controls, as hippocampal volume declines with age[50].Thus, age-matching might have revealed a higher postpartum hippocampal volume, compared to controls, but not a significant reduction. Intriguingly, we observed a positive association between subclinical depressive symptoms and hippocampal volume, which is in the opposite direction of what we would expect from studies in clinically depressed non-postpartum patients [34]. However, it is normal for hippocampus to decrease in volume across pregnancy and the postpartum period, as it goes through a highly plastic phase that results in a remodeling of the brain [60,61]. Thus, a *high* postpartum hippocampal volume may reflect maladaptive plasticity across pregnancy, which limits the ability to adjust appropriately to the perinatal transition, and thus increases the risk for mental distress. This may be related to altered 5-HT4R availability, as 5-HT4R is believed to have antidepressant properties that, in part, are mediated through increased neuroplasticity and cell proliferation in hippocampus [25]. Our data supports this notion since interaction analyses indicated that high 5-HT4R in all regions, but especially hippocampus, in interaction with a large decrease was associated with a smaller volume. If we consider 5-HT4R a marker for capacity for plasticity, a large E2 decrease combined with adaptive/high plasticity may lead to a smaller hippocampus in healthy perinatal transitions. In line with this, a previous study that included this group of women, indicated that women with a large perinatal decrease in estradiol had fewer distress symptoms [15]. Intriguingly, also studies have shown that genomic estradiol sensitivity markers that increase the risk for PND involves genes that code for components in the serotonin system [10,62,63].

Our results indicate that hippocampal volume remains within the normal range for healthy postpartum women, but that the regulatory mechanisms may be substantially different from those at work outside the postpartum state. Further, we speculate that women with a higher postpartum hippocampal volume may have undergone maladaptive hippocampal remodeling across peripartum, which limits their ability to adapt to the postpartum period and may in turn trigger mental distress. Additionally, 5-HT4R in interaction with change in E2 may play a role in postpartum hippocampal plasticity.

Methodological considerations

This study was designed to target the ante- to postpartum transition, however brain scans of pregnant women are not possible due to fetus safety, thus we were not able to illuminate within-participant trajectories for the imaging part. Further, despite the best of our efforts to include also distressed women in the study, mentally robust were more likely to accept the invitation, especially for the PET scans, which were more demanding. Thus, we generally have a healthy sample with pronounced floor effects on the two depression scales, which limits our ability to extrapolate to clinical cohorts with more symptoms or with manifest perinatal depressive episodes. Controls were significantly younger than the postpartum group. Although we age-adjusted hippocampal volume and 5-HT4R, we may have overlooked a relevant group difference, which would have been detectable with age-matched controls. As both 5-HT4R and hippocampal volume declines with age, we are however only likely to have missed a significantly higher volume or 5-HT4R brain binding in the postpartum women, not a lower [50,64,65]. Our power calculations were based on data not directly comparable to the study group, which may not translate directly to the postpartum state, and consequently we may have been underpowered to detect any group differences. Finally, we acknowledge that we have a small sample size, vulnerable to noise, and our results, especially the within-group interaction analyses, should be interpreted with caution due to the limited power.

Conclusions

We show that healthy controls and postpartum women do not differ in terms of 5-HT4R and hippocampal volume, which suggests that the brain generally adapts well to perinatal transition and the postpartum state. Further, subclinical levels of mental distress were positively associated with hippocampal volume in postpartum women. We speculate that a larger postpartum hippocampal volume may reflect maladaptive hippocampal plasticity across the perinatal transition, which increases the vulnerability to subclinical levels of mental distress. Further, our data indicated that 5-HT4R in interaction with E2 may play an important role in postpartum plasticity. Importantly, our results reflect variations within the healthy spectrum and future studies must elucidate if this translates to mechanisms at work in clinically depressed postpartum women

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Conflict of interest

VGF declares that she has received honorarium as a consultant for Sage Therapeutics and lectures for Lundbeck Pharma A/S.

References

1 1 Robertson E, Grace S, Wallington T, Stewart DE. Antenatal risk factors for postpartum depression: A synthesis of recent literature. *Gen Hosp Psychiatry* 2004; **26**: 289–295.

2 Okun ML, Luther J, Prather AA, Perel JM, Wisniewski S, Wisner KL. Changes in sleep quality, but not hormones predict time to postpartum depression recurrence. *J Affect Disord* 2011; **130**: 378–384.

3 Reck C, Stehle E, Reinig K, Mundt C. Maternity blues as a predictor of DSM-IV depression and anxiety disorders in the first three months postpartum. *J Affect Disord* 2009; **113**: 77–87.

4 Beck CT. Predictors of postpartum depression: An update. *Nurs Res* 2001; **50**: 275–285.

5 Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal depression: A systematic review of prevalence and incidence. *Obstet Gynecol* 2005; **106**: 1071–1083.

6 Meltzer-Brody S, Boschloo L, Jones I, Sullivan PF, Penninx BW. The EPDS-Lifetime: Assessment of lifetime prevalence and risk factors for perinatal depression in a large cohort

of depressed women. *Arch Womens Ment Health* 2013; **16**: 465–473.

7 American Psychiatric Association. DSM-5 Task Force. *Diagnostic and statistical manual of mental disorders : DSM-5.* American Psychiatric Association, 2013.

8 Putnam KT, Wilcox M, Robertson-Blackmore E, Sharkey K, Bergink V, Munk-Olsen T *et al.* Clinical phenotypes of perinatal depression and time of symptom onset: analysis of data from an international consortium. *The Lancet Psychiatry* 2017; **4**: 477–485.

9 wilson M, Morganti AA, Zervoudakis I, Letcher RL, Romney BM, Von Oeyon P *et al.* Blood pressure, the renin-aldosterone system and sex steroids throughout normal pregnancy. *Am J Med* 1980; **68**: 97–104.

10 Mehta D, Newport DJ, Frishman G, Kraus L, Rex-Haffner M, Ritchie JC *et al.* Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. *Psychol Med* 2014; **44**: 2309–2322.

11 McNeilly AS. Breastfeeding and the suppression of fertility. *Food Nutr Bull* 1996; **17**: 340–345.

12 Frokjaer VG, Pinborg A, Holst KK, Overgaard A, Henningsson S, Heede M *et al.* Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: A positron emission tomography study. *Biol Psychiatry* 2015; **78**: 534–543.

13 Sanjuán J, Martin-Santos R, Garcia-Esteve L, Carot JM, Guillamat R, Gutierrez-Zotes A *et al.* Mood changes after delivery: Role of the serotonin transporter gene. *Br J Psychiatry* 2008; **193**: 383–388.

14 Tavares Pinheiro R, Monteiro Da Cunha Coelho F, Azevedo Da Silva R, Amaral Tavares Pinheiro K, Pierre Oses J, De Ávila Quevedo L *et al.* Association of a serotonin transporter gene polymorphism (5-HTTLPR) and stressful life events with postpartum depressive symptoms: A population-based study. *J Psychosom Obstet Gynecol* 2013; **34**: 29–33.

15 Borgsted C, Høgh S, Fonnesbech-Sandberg L, Høgsted ES, Ekelund K, Albrechtsen CK *et al.* P.0511 The role of sex steroids and serotonin brain dynamics in perinatal mental health. *Eur Neuropsychopharmacol* 2021; **53**: S376–S377.

16 Carpenter LL, Anderson GM, Siniscalchi JM, Chappell PB, Price LH. Acute changes in cerebrospinal fluid 5-HIAA following oral paroxetine challenge in healthy humans. *Neuropsychopharmacology* 2003; **28**: 339–347.

17 Y S, ME B, JG C. Correlated reductions in cerebrospinal fluid 5-HIAA and MHPG concentrations after treatment with selective serotonin reuptake inhibitors. *J Clin Psychopharmacol* 1997; **17**: 11–14.

18 De Bellis MD, Geracioti TD, Altemus M, Kling MA. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. *Biol Psychiatry* 1993; **33**: 636–641.

19 Potter WZ, Scheinin M, Golden RN, Rudorfer M V., Cowdry RW, Calil HM *et al.* Selective Antidepressants and Cerebrospinal Fluid: Lack of Specificity on Norepinephrine and Serotonin Metabolites. *Arch Gen Psychiatry* 1985; **42**: 1171–1177.

20 Lu NZ, Eshleman AJ, Janowsky A, Bethea CL. Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution, and function in female macaques. *Mol Psychiatry* 2003; **8**: 353–360.

21 Suda S, Segi-Nishida E, Newton SS, Duman RS. A Postpartum Model in Rat: Behavioral and Gene Expression Changes Induced by Ovarian Steroid Deprivation. *Biol Psychiatry* 2008; **64**: 311–319.

22 Sharp T, Barnes NM. Central 5-HT receptors and their function; present and future. Neuropharmacology. 2020; **177**: 108155.

23 Haahr ME, Fisher PM, Jensen CG, Frokjaer VG, McMahon B, Madsen K *et al.* Central 5-HT4 receptor binding as biomarker of serotonergic tonus in humans: A [11C]SB207145 PET study. *Mol Psychiatry* 2014; **19**: 427–432.

24 Fisher PM, Holst KK, Mc Mahon B, Haahr ME, Madsen K, Gillings N *et al.* 5-HTTLPR status predictive of neocortical 5-HT4binding assessed with [11C]SB207145 PET in humans. *Neuroimage* 2012; **62**: 130–136.

25 Murphy SE, De Cates AN, Gillespie AL, Godlewska BR, Scaife JC, Wright LC *et al.* Translating the promise of 5HT 4 receptor agonists for the treatment of depression. Psychol. Med. 2021; **51**: 1111–1120.

26 Hampson E, Morley EE. Estradiol concentrations and working memory performance in women of reproductive age. *Psychoneuroendocrinology* 2013; **38**: 2897–2904.

27 Blackmore ER, Gustafsson H, Gilchrist M, Wyman C, O'Connor TG. Pregnancy-related anxiety: Evidence of distinct clinical significance from a prospective longitudinal study. *J*

Affect Disord 2016; **197**: 251–258.

28 Koehler-Forsberg K, Dam V, Joergensen A, Landman E, Larsen SV, Poulsen AS *et al.* P.352 Patients with major depressive disorder have lower cerebral serotonin 4 receptor binding than healthy controls. *Eur Neuropsychopharmacol* 2019; **29**: S251–S252.

29 Larsen S V., Köhler-Forsberg K, Dam VH, Poulsen AS, Svarer C, Jensen PS *et al.* Oral contraceptives and the serotonin 4 receptor: a molecular brain imaging study in healthy women. *Acta Psychiatr Scand* 2020; **142**: 294–306.

30 Hagena H, Manahan-Vaughan D. The serotonergic 5-HT4 receptor: A unique modulator of hippocampal synaptic information processing and cognition. *Neurobiol Learn Mem* 2017; **138**: 145–153.

31 Pawluski JL, Lambert KG, Kinsley CH. Neuroplasticity in the maternal hippocampus: Relation to cognition and effects of repeated stress. *Horm Behav* 2016; **77**: 86–97.

32 Pawluski JL, Brummelte S, Barha CK, Crozier TM, Galea LAM. Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. *Front Neuroendocrinol* 2009; **30**: 343–357.

33 Hoekzema E, Barba-Müller E, Pozzobon C, Picado M, Lucco F, García-García D *et al.* Pregnancy leads to long-lasting changes in human brain structure. *Nat Neurosci* 2017; **20**: 287–296.

34 Schmaal L, Veltman DJ, Van Erp TGM, Smann PG, Frodl T, Jahanshad N *et al.* Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 2016; **21**: 806–812.

35 Sheehan D V., Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E *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* 1998; **59**: 22–33.

36 Knudsen GM, Jensen PS, Erritzoe D, Baaré WFC, Ettrup A, Fisher PM *et al.* The Center for Integrated Molecular Brain Imaging (Cimbi) database. *Neuroimage* 2016; **124**: 1213–1219.

37 Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C *et al.* Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; **33**: 341–355.

38 Puonti O, Iglesias JE, Van Leemput K. Fast and sequence-adaptive whole-brain segmentation using parametric Bayesian modeling. *Neuroimage* 2016; **143**: 235–249.
39 Fischl B. FreeSurfer. Neuroimage. 2012; **62**: 774–781.
40 Ashburner J. SPM: A history. *Neuroimage* 2012; **62**: 791–800.
41 Madsen K, Marner L, Haahr M, Gillings N, Knudsen GM. Mass dose effects and in vivo affinity in brain PET receptor studies - a study of cerebral 5-HT 4 receptor binding with [11C]SB207145. *Nucl Med Biol* 2011; **38**: 1085–1091.
42 Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbøl S, Frøkjær VG *et al.* MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* 2005; **24**: 969–979.
43 Marner L, Gillings N, Comley RA, Baaré WFC, Rabiner EA, Wilson AA *et al.* Kinetic modeling of11C-SB207145 binding to 5-HT4 receptors in the human brain in vivo. *J Nucl Med* 2009; **50**: 900–908.
44 Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage* 1996; **4**: 153–158.
45 Smith-Nielsen J, Matthey S, Lange T, Væver MS. Validation of the Edinburgh Postnatal Depression Scale against both DSM-5 and ICD-10 diagnostic criteria for depression. *BMC Psychiatry* 2018; **18**: 393.
46 HAMILTON M. Development of a Rating Scale for Primary Depressive Illness. *Br J Soc Clin Psychol* 1967; **6**: 278–296.
47 Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. *J Affect Disord* 2001; **66**: 159–164.
48 Frederiksen H, Johannsen TH, Andersen SE, Albrethsen J, Landersoe SK, Petersen JH *et al.* Sex-specific Estrogen Levels and Reference Intervals from Infancy to Late Adulthood Determined by LC-MS/MS. *J Clin Endocrinol Metab* 2020; **105**: 754–768.
49 Weikop P, Kehr J, Scheel-Krüger J. Reciprocal effects of combined administration of serotonin, noradrenaline and dopamine reuptake inhibitors on serotonin and dopamine levels in the rat prefrontal cortex: the role of 5-HT 1A receptors. *J Psychopharmacol* 2007; **21**: 795–804.

50 Nobis L, Manohar SG, Smith SM, Alfaro-Almagro F, Jenkinson M, Mackay CE *et al.* Hippocampal volume across age: Nomograms derived from over 19,700 people in UK Biobank. *NeuroImage Clin* 2019; **23**: 101904.

51 Sacher J, Rekkas PV, Wilson AA, Houle S, Romano L, Hamidi J *et al.* Relationship of monoamine oxidase-A distribution volume to postpartum depression and postpartum crying. *Neuropsychopharmacology* 2015; **40**: 429–435.

52 Sacher J, Wilson AA, Houle S, Rusjan P, Hassan S, Bloomfield PM *et al.* Elevated brain monoamine oxidase A binding in the early postpartum period. *Arch Gen Psychiatry* 2010; **67**: 468–474.

53 Moses-Kolko EL, Wisner KL, Price JC, Berga SL, Drevets WC, Hanusa BH *et al.* Serotonin 1A receptor reductions in postpartum depression: a positron emission tomography study. *Fertil Steril* 2008; **89**: 685–692.

54 Lokuge S, Frey BN, Foster JA, Soares CN, Steiner M. Depression in women: Windows of vulnerability and new insights into the link between estrogen and serotonin. *J Clin Psychiatry* 2011; **72**: e1563–e1569.

55 Deecher D, Andree TH, Sloan D, Schechter LE. From menarche to menopause: Exploring the underlying biology of depression in women experiencing hormonal changes. Psychoneuroendocrinology. 2008; **33**: 3–17.

56 Kuijper EAM, Ket JCF, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: A systematic review. Reprod. Biomed. Online. 2013; **27**: 33–63.

57 Williams RB, Marchuk DA, Gadde KM, Barefoot JC, Grichnik K, Helms MJ *et al.* Serotonin-related gene polymorphisms and central nervous system serotonin function. *Neuropsychopharmacology* 2003; **28**: 533–541.

58 Mårtensson B, Wägner A, Beck O, Brodin K, Montero D, Åsberg M. Effects of clomipramine treatment on cerebrospinal fluid monoamine metabolites and platelet H-imipramine binding and serotonin uptake and concentration in major depressive disorder. *Acta Psychiatr Scand* 1991; **83**: 125–133.

59 Martínez-García M, Paternina-Die M, Barba-Müller E, de Blas DM, Beumala L, Cortizo R *et al.* Do pregnancy-induced brain changes reverse? The brain of a mother six years after parturition. *Brain Sci* 2021; **11**: 1–14.

60 Pawluski JL, Hoekzema E, Leuner B, Lonstein JS. Less Can Be More: Fine Tuning the Maternal Brain. *Neurosci Biobehav Rev* 2021. doi:10.1016/j.neubiorev.2021.11.045.

61 Been LE, Sheppard PAS, Galea LAM, Glasper ER. Hormones and neuroplasticity: A lifetime of adaptive responses. *Neurosci Biobehav Rev* 2021. doi:10.1016/j.neubiorev.2021.11.029.

62 Guintivano J, Arad M, Gould TD, Payne JL, Kaminsky ZA. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. *Mol Psychiatry* 2014; **19**: 560–567.

63 Mehta D, Rex-Haffner M, Sondergaard HB, Pinborg A, Binder EB, Frokjaer VG. Evidence for oestrogen sensitivity in perinatal depression: Pharmacological sex hormone manipulation study. *Br J Psychiatry* 2019; **215**: 519–527.

64 Marner L, Gillings N, Madsen K, Erritzoe D, Baaré WFC, Svarer C *et al.* Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. *Neuroimage* 2010; **50**: 855–861.

65 Madsen K, Haahr MT, Marner L, Keller SH, Baaré WF, Svarer C *et al.* Age and sex effects on 5-HT 4 receptors in the human brain: A 11 CSB207145 PET study. *J Cereb Blood Flow Metab* 2011; **31**: 1475–1481.

Figure legends

Figure 1 Group differences in A) age-adjusted 5-HT4R binding in neostriatum (residuals) and B) ag- adjusted hippocampal volume (residuals). We observed no significant difference between healthy controls and postpartum women in terms of 5-HT4R binding (p = 0.63 for neostriatum) or hippocampal volume (p = 0.61).

Figure 2. Adjusted for parity and ΔE2, mean hippocampal volume was associated on a borderline significant level with A) HAMD-17 (p = 0.04) and B) EPDS (p = 0.06).

Figure 3. Interaction effect between 5-HT4R binding in neostriatum and ΔE2 on hippocampal volume. High 5-HT4R brain binding in interaction with a small decrease in E2 was associated with a larger postpartum hippocampal volume. In neostriatum, this interaction was significant (p = 0.02). Note that a large negative value on the x-axis corresponds to a large peripartum decrease in E2. For this two-dimensional visualization of the interaction, 5-HT4R binding in neostriatum was dichotomized with a median split. The resulting high (solid line) and low (dashed line) binding groups are therefore arbitrary. Grey zones illustrate 95% CIs.

Tables

Table 1. Characteristics for postpartum women. All 31 completed structural MR scans, 23 women also completed ^{11}C-SB207145 PET.

	MR scanned (N = 31)		PET scanned (N = 23)	
	Antepartum	***Week five***	***Antepartum***	***Week five***

Age (years)	34 (4) [a]	-	34 (4) [a]	-
Parity (multiparous / primiparous)	-	16/15	-	11/12
Sex of child (male /female)		16/15		12/11
5-HTTLPR LL/S-carrier	8 / 23	-	5 / 18	-
MDI	7.16 (4.38) [a]	5.59 (3.24) [a]	7.70? (4.52) [a]	5.67 (3.23) [a]
HAMD-17	-	0.81 (1.8) [a]	-	1.04 (2.03) [a]
EPDS	-	3.93 (2.73) [a]	-	3.71 (2.8) [a]
E2 in pmol/L	83399 (30356) [a]	106 (231) [a]	85063 (30548) [a]	59 (57) [a]
ΔE2 in pmol/L	-	-83293 (30293) [a]	-	-85004 (30550) [a]
5-HIAA in fmol/10µL	1071.37 (420.47) [a]	-	1058.22 (449.49) [a]	-
[a] Mean (SD)				

Figures

Figure 1.

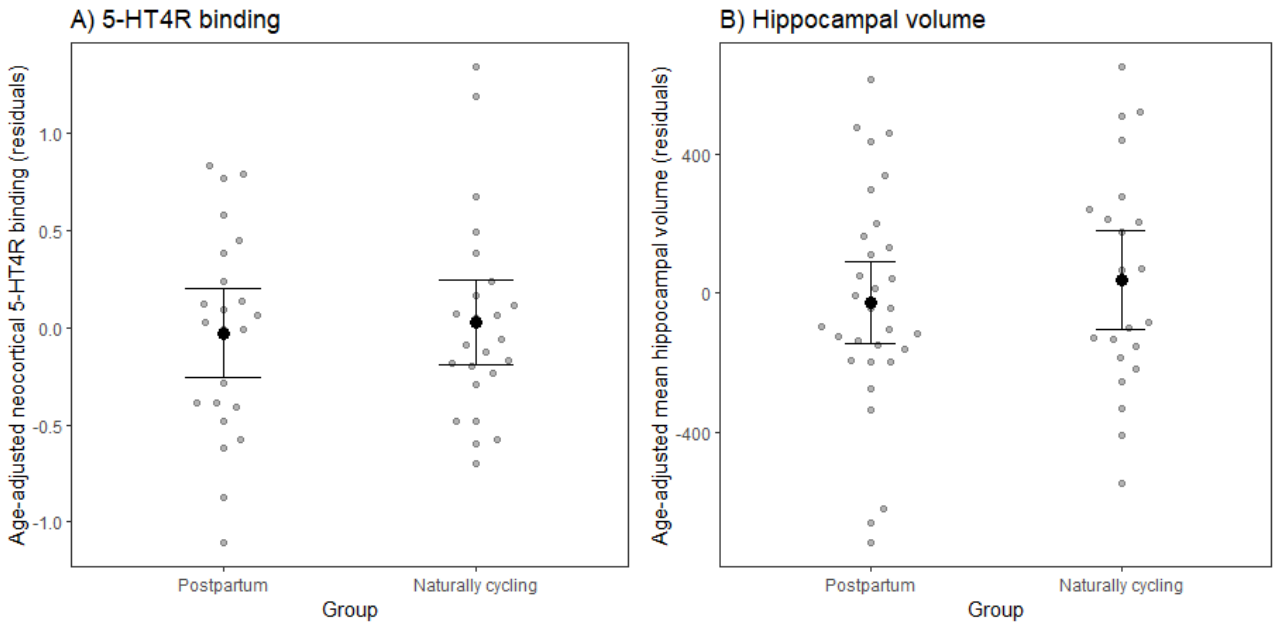


Figure 2.

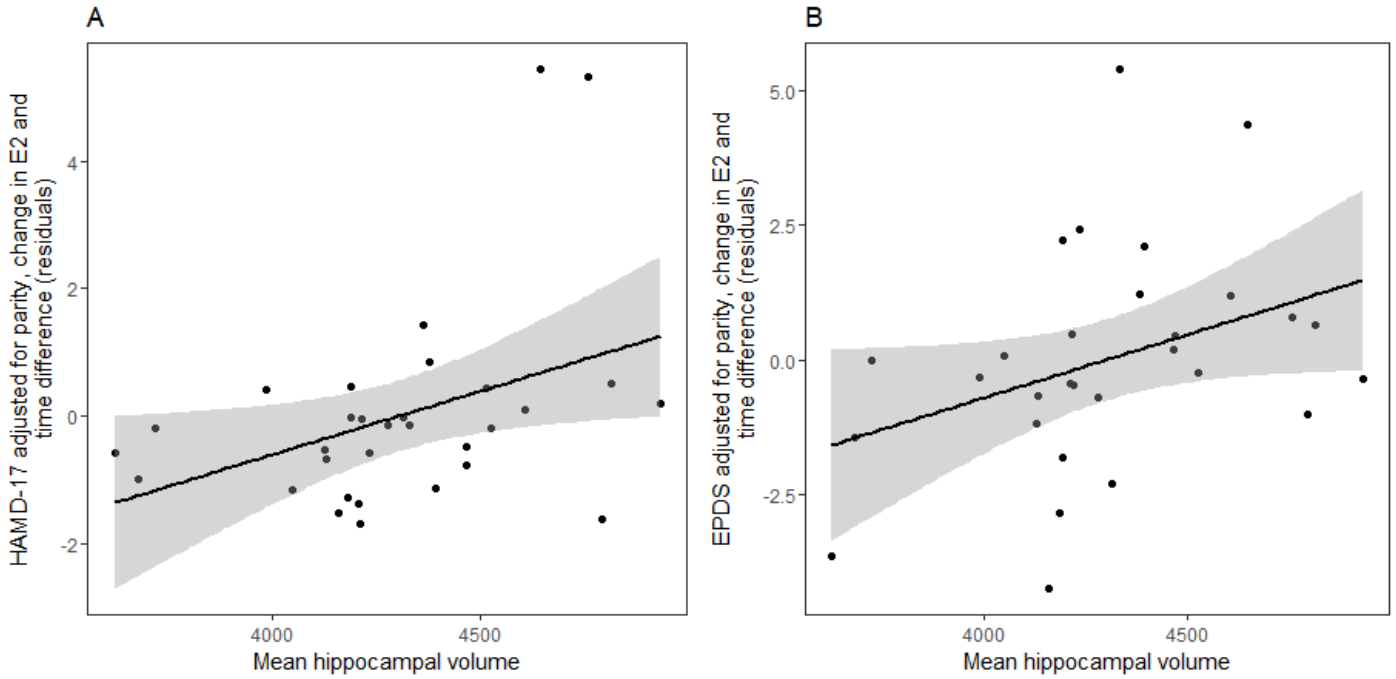
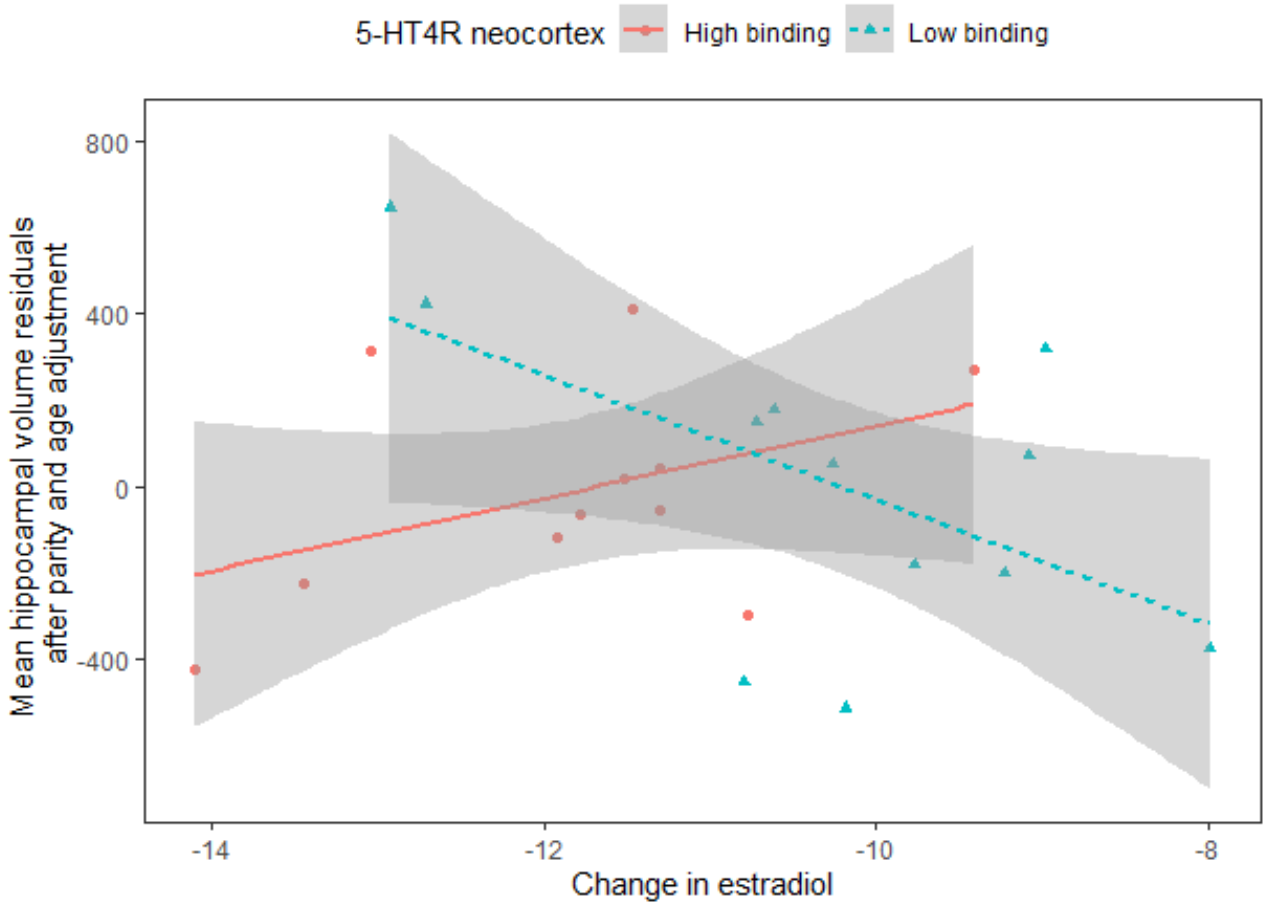


Figure 3.


5-HT4R neocortex
High binding
Low binding
Mean hippocampal volume residuals after parity and age adjustment
800
400
0
-400
-14
-12
-10
-8
Change in estradiol

Supplementary materials

Model selction with AIC

Since MR imaging was often conducted on a different day than the PET scans and blood samples (the PET and blood samples were always acquired on the same day), the time difference was considered a potential covariate. However, it did not improve the model fit evaluated with AIC:
AIC, model without time difference: 333.6202
AIC, model with time difference: 335.5285