Oral contraceptives and the serotonin 4 receptor
a molecular brain imaging study in healthy women

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Objective: Sex steroid hormones potently shape brain functions, including those critical to maintain mental health such as serotonin signaling. Use of oral contraceptives (OCs) profoundly changes endogenous sex steroid hormone levels and dynamics. Recent register-based studies show that starting an OC is associated with increased risk of developing depression. Here, we investigate whether use of OCs in healthy women is associated with a marker of the serotonin system in terms of serotonin 4 receptor (5-HT4R) brain imaging.

Methods: [11C]SB207145-PET imaging data on 53 healthy women, of whom 16 used OCs, were available from the Cimbi database. We evaluated global effects of OC use on 5-HT4R binding in a latent variable model based on 5-HT4R binding across cortical and subcortical regions.

Results: We demonstrate that OC users have 9–12% lower global brain 5-HT4R binding potential compared to non-users. Univariate region-based analyses (pallidostriatum, caudate, hippocampus, amygdala, anterior cingulate cortex, and neocortex) supported the global effect of OC use with the largest difference present in the hippocampus (−12.8% (95% CI [−21.0; −3.9]), $P_{\text{corrected}} = 0.03$).

Conclusion: We show that women who use OCs have markedly lower brain 5-HT4R binding relative to non-users, which constitutes a plausible molecular link between OC use and increased risk of depressive episodes. We propose that this reflects a reduced 5-HT4R gene expression, possibly related to a blunted ovarian hormone state among OC users.

Significant outcomes

- Healthy women who use oral contraceptives have 9–12% lower global brain serotonin 4 receptor binding potential compared to non-users.
- The lower serotonin 4 receptor binding potential provides a plausible molecular mechanism by which oral contraceptive use can be associated with increased risk of developing depression.
- Compared to previous studies, the effect size of oral contraceptive use is almost twice as big as the effect size of selective serotonin reuptake inhibitors (SSRIs) on serotonin 4 receptor binding potential. This raises the question if concomitant oral contraceptive use affects SSRI antidepressant efficacy.
Limitations

- The study is cross-sectional, and thus, causality cannot be inferred.
- We only included healthy women, which increases the risk of 'survival effect' in the oral contraceptive user group, and hence potentially reduces the effect size of oral contraceptive use on serotonin 4 receptor binding potential.

Introduction

More than 100 million women worldwide use oral contraceptives (OCs) (1,2), and indeed, they have profound implications for women’s health including the avoidance of unwanted pregnancies and abortions as well as menstrual cycle disturbances (3). However, not all women tolerate OCs; some are at excessive risk of thromboembolic events (4), and others are forced to discontinue OC use because of experienced side effects including mood deterioration (5). Recently, register-based epidemiological work has shown that starting on hormonal contraceptives is associated with an increased risk of developing a depressive episode, based on either subsequent first-time use of an antidepressant or receiving a first diagnosis of major depressive disorder (MDD) (6, 7), including the severe cases that involve suicide (8). Additionally, these studies point out that adolescents were at higher risk relative to women older than 20 years, which has since been replicated in an independent prospective cohort study (9). Further, another study suggests that first time use of OCs in adolescence compared to in adulthood is associated with longer lasting vulnerability to MDD through adulthood (10). The literature, however, shows conflicting results as smaller studies have reported positive or no mood effect of OC use (11). Nevertheless, as adolescent females start on OCs at an increasingly young age (12) their brains are more susceptible to the potential impact of exogenous hormones as they go through critical stages of brain maturation (13). This emphasizes the importance of investigating how OCs may affect brain biology, which is putatively critical to mental health.

It remains unknown why use of OCs may increase the risk of a depressive episode, at least in a subgroup of women and how these women may be identified. Previous findings indicate that OC use can affect brain structure (14) and function (15), which is important for affective control such as fear processing (16), and further OC use distorts the hypothalamic–pituitary–adrenal axis into a state comparable to chronic stress (16, 17). However, to our knowledge, the effects of OC use on neurotransmission, including the serotonin brain system, are very scarcely studied.

OCs suppress the hypothalamic–pituitary–gonadal hormonal axis, which drives the reproductive system, and consequently downregulates endogenous ovarian sex steroid hormone production and disrupts follicular maturation and ovulation (18). This suppression is profound and clearly visible by ultrasound where OC use may halve the size of the ovaries (18). The synthetic OC steroids, that is, ethinyl estradiol and a generation-specific progestin, substitute the endogenous hormones only to some extent; in OC users, the blood concentration of total estrogen (exogenous + endogenous) is considerably low compared to in non-users, but when considering the relative binding affinity for the estradiol receptor (ER), the average daily estrogen exposure compares to the natural exposure. In contrast, the progesterone exposure can be eight-fold increased (19). However, the synthetic steroids upregulate the level of sex hormone-binding globulin, which reduces the freely available sex steroid hormones (20). Such changes in sex steroid milieu can shape human brain biology, including the serotonergic system (21, 22), which is key in maintaining mental health and is critically involved in the risk for and treatment of MDD (23). Estradiol, in particular, directly targets serotonergic neurons and affects key features of the serotonin signaling system (21) including gene expression of the serotonin transporter, the main regulator of synaptic serotonin (24).

One of the most recent advances in serotonin brain positron emission tomography (PET) imaging is the quantification of the postsynaptic serotonin 4 receptor (5-HT4R) by the \[^{11}C\]SB207145-PET radioligand (25). The 5-HT4R is itself a potential target in MDD as 5-HT4R stimulation has fast acting antidepressant-like properties (26), and further, it is distributed abundantly throughout subcortical regions (27) along with sex hormone receptors (22). Animal and human studies suggest that 5-HT4R binding is inversely
correlated to serotonergic brain tonus (28–30). Thus, 5-HT4R brain binding constitutes an informative marker of serotonergic neurotransmission and provides an opportunity to elucidate a potential molecular link between OC use and risk of developing MDD.

Aims of the study

In this cross-sectional study, we investigate whether healthy women who use oral contraceptives differ relative to non-users in terms of global serotonin 4 receptor binding in the brain.

Material and Methods

Participants

[11C]SB207145-PET neuroimaging binding potential data and associated data from a total of 74 healthy women were extracted from the Center for Integrated Molecular Brain Imaging (Cimbi) database (1). Participants had been recruited for different neuroimaging projects between 2006 and 2018. All projects had been approved by the Ethics Committee of Copenhagen and Frederiksberg or of RegionH, Denmark ((KF)01-274821, (KF)01-2006-20, H-15004506, H-1-2010-085, H-4-2012-105, H-6-2014-057, H-15017713). Participants had normal physical and neurological examinations as well as unremarkable brain magnetic resonance imaging (MRI) scans. Women were not included if they were pregnant or breastfeeding and had a significant medical history including psychiatric or neurological diseases or dependence of drugs or alcohol. Of the 74 women from the database, we included 61 women with available information about hormonal contraceptive use, younger than 50 years (i.e., presumably premenopausal), and body mass index (BMI) < 30 kg/m², as large deposits of fat tissue produce estrogen (31). Further, one participant was excluded because of polycystic ovary syndrome, two because of PET-tracer doses that exceeded our upper limit of cold mass injected (32), and four because of recent (<2 months) discontinuation of OCs or vaginal ring (33). Finally, one OC user was excluded because her progesterone levels were 24.5 nmol/l, indicating non-compliance (18). Thus, 53 healthy women were available for analyses, of which 16 used OCs.

Women using a hormonal intrauterine device (IUD) were included as non-users because the majority are expected to have a normal ovulatory cycle (34). In a supplementary analysis, we tested if they differed from other non-users in terms of 5-HT4R binding potentials, which was not the case (Table S1). Screening for MDD and mood disturbances as well as other psychopathology was done using the 10 item Major Depression Inventory (MDI) questionnaire (35), the Cohen’s Perceived Stress Scale, the Profile of Mood States—Total Mood Disturbance (POMS-TMD), and the Symptom Checklist-92 Global Severity Index (SCL-92 GSI). Familial risk for MDD was acquired through the Family History Assessment Module (FHAM) questionnaire (36). Neuroticism, a personality trait associated with MDD (37), was assessed via the Danish version of the five-factor NEO Personality Inventory-Revised (NEO PI-R) (38). Educational level was scored via a 5-point Likert scale, scoring one if having no vocational degree and five if having more than four years of higher academic education. Information about relationship status was acquired on the scan date via interview.

Oral contraceptives

Information about use of hormonal contraceptives was achieved by face-to-face interview at the day of PET scan and by written questionnaires including a question about the type of OC used. Those with missing information were contacted at a later time point by e-mail or phone to specify type of OC used and in two cases if OC was used at time of the scan. The type of OC is known for 12 out of the 16 users, all of which were ‘combined OCs’; 2nd and 3rd generation combined OCs containing ethinylestradiol in combination with levonorgestrel or gestodene (Table S2).

Plasma levels of sex steroid hormones

Measurements of sex steroid hormones were included in the standard program for healthy volunteers as of March 2011 (1), and thus, data were available for 42 (11 OC users and 31 non-users) of the 53 women. However, because of changes in hospital analysis method of plasma sex steroids (change of antibody reagents from Estradiol II/Progesterone II to Elecsys® Estradiol III/Progestosterone III on Cobas 8000 e602 module, Roche), 11 progesterone measurements were excluded because the analysis methods were non-compatible resulting in 31 included samples (9 OC users and 22 non-users). Estradiol measurements were compatible between the analysis methods by use of a conversion factor (validation reports can be obtained upon request). The new method resulted in a higher lower detection limit for the plasma estradiol level (0.09 nmol/l compared to 0.04 nmol/l). For the descriptive statistics, the estradiol samples
below the detection limit (10 out of the 42 measurements) were imputed to the respective lower limit value and an extended version of Wilcoxon test, the Gehan test (39), was performed to compare the plasma estradiol concentrations between the groups.

Imaging

The acquisition of scans and image analysis is detailed elsewhere (25). A brief overview is presented here. High-resolution T1-weighted structural MR images were acquired on three different scanners: Siemens MAGNETOM 3T MRI scanners (Prisma \((n = 41)\), Verio \((n = 2)\), and Trio \((n = 10)\)). MR images were segmented into cerebrospinal fluid, gray matter, and white matter by use of Statistical Parametric Mapping (SPM5 or SPM8, The Wellcome Centre for Human Neuroimaging, UCL, London, UK). Individual PET and MR images for each subject were co-registered using the automatic methods from SPM5 or SPM8. Regions were automatically delineated using each subject’s MR image via the user-independent algorithm in the Pvelab software package (40). Regions of interest included hippocampus, amygdala, anterior cingulate cortex (ACC), neocortex, pallidostriatum, and caudate. The former four regions were chosen because of their high expression level of sex hormone receptors and sensitivity to hormonal changes (22), and the latter two because they have a high density of 5-HT4Rs (27).

\[^{11}\text{C}]SB207145\)-PET scans were acquired either on an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA) \((n = 6)\) with an approximate in-plane resolution of 6 mm, or a High-Resolution Research Tomograph (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA) \((n = 47)\) with a 1.4-mm resolution (centre of field of view). All scans were obtained from a 120-minute dynamic acquisition starting immediately after bolus injection of \[^{11}\text{C}]SB207145\). Framing of the dynamic scans and procedures for head motion correction are described elsewhere (28). Non-displaceable binding potentials \((BP_{ND})\) were estimated using the simplified reference tissue model with cerebellum as reference tissue (25) using an in-house batch-mode algorithm validated against kinetic modeling with PMOD software version 2.95 (PMOD Inc., Zurich, Switzerland, http://www.pmod.com/web).

For illustrative purposes, PXMOD was used to generate single-subject voxel-based parametric \(BP_{ND}\) images. For this, we only used the HRRT PET images \((n = 47)\), which were smoothed across adjacent voxels by applying a Gaussian filter within each time frame of the dynamic PET image and were normalized into standard space with SPM12 and visualized in FreeView (FreeSurfer software, http://surfer.nmr.mgh.harvard.edu/, v6.0.0). A percent difference map was generated by fitting our multiple linear regression model (described below) at each voxel, with mean-centered continuous covariates, and dividing the voxel-specific marginal OC use effect by its model intercept, multiplied by 100.

Statistics

We evaluated whether OC use is associated with a global pattern of 5-HT4R brain binding in a linear latent variable model (LVM) framework where the effect of OC use is mediated through a single latent variable across brain regions of low (neocortex), intermediate (ACC, hippocampus, and amygdala), and high (pallidostriatum and caudate) 5-HT4R binding (27). The latent variable enables to model the large correlation in 5-HT4R binding between brain regions (41). Covariates included age, scanner type (GE-Advance PET scanner vs. HRRT Siemens PET scanner), injected \[^{11}\text{C}]SB207145\) mass per kg bodyweight, and familial risk for MDD (as binary factor, i.e., having none vs. one or more first-degree relatives with MDD), which are known to influence 5-HT4R PET measurements (42–44). This adjustment was done independently in each region, that is, not mediated through the latent variable.

To support the LVM and provide a more conceptually accessible description of the effect of OC use on 5-HT4R brain binding, we also present a univariate multiple linear regression analysis of the association between OC use and 5-HT4R \(BP_{ND}\) in the six regions of interest. These models included the same covariates as the LVM. In both models, 5-HT4R \(BP_{ND}\) values were log-transformed prior to modeling and the estimated OC effect on the regional binding is expressed as a percent difference in 5-HT4R binding between OC users and non-users.

Diagnostic tools were used to assess the adequacy of the model’s assumptions. In case of unequal residual variance, the linear model was re-estimated while allowing for different residual variances between the groups. For the LVM, the specification of the covariance structure was assessed using a chi-squared test. When suboptimal specification was indicated (chi-square \(P < 0.05\), score tests (see section 6.2.4 in (45)) were used to identify covariance parameters to include in the model. This procedure was iteratively performed until the
chi-squared test was no longer significant (chi-square $P > 0.05$). Two-sided statistical tests were used, and $P < 0.05$ was considered statistically significant. When testing the OC effect on each region separately, $P$-values and confidence intervals were adjusted for multiple comparisons using the single-step Dunnett’s procedure (46). Statistical analyses were performed in R (47), and the lava package (45) was used to create the LVM.

### Results

**Study population profile**

Demographic, psychometric, endogenous hormone profiles, and PET parameters of the study population are shown in Table 1. The groups (OC user vs. non-user) were similar in age, BMI, educational level, relationship status, and neuroticism score. However, the OC user group tended to have more first-degree relatives with MDD ($P = 0.05$). Since this may be a relevant confounder (43), accordingly, it was taken into consideration in the regression model evaluating differences in 5-HT4R between groups. The study population scored within the range of a healthy population in terms of no signs of depression; that is, MDI averages 4.8 (range 0–12) (48), and no signs of other psychopathology evaluated by the global severity index (SLC-92 GSI) (49). Plasma estradiol levels in OC users (mean ± standard deviation: 0.09 ± 0.06 nmol/l) were lower relative to non-users (mean ± standard deviation: 0.46 ± 0.40 nmol/l), $P < 0.001$. Progesterone levels were also reduced in OC users ($P = 0.04$).

**Effects of OC use on serotonin 4 receptor binding**

Support for the LVM structure was evidenced by all region-specific bindings loading onto the latent variable ($P < 0.001$). To satisfy the chi-squared test, two covariance parameters (between neocortex and ACC and between hippocampus and amygdala) were added to the LVM, which only had a minor impact on the estimate of the effect of OC use. No additional parameter modeling region-specific effect of OC use was found necessary. The LVM identified a global pattern of association between OC use and 5-HT4R binding; the effect of OC use was mediated by the global latent variable with a negative association between OC use and the individual 5-HT4R latent levels ($\gamma = -0.099$, $P = 0.0039$, Fig. 1). Regional loadings varied, corresponding to an effect of OC use of −9.4% to −11.7% in BPND. The global effect is illustrated in parametric maps in Fig. 2. No gross deviation from the normality assumption was observed.

The univariate region-based analyses supported the global effect of OC use as depicted in Table 2. However, amygdala, which was significantly associated with the shared latent variable in the LVM, did not display statistical significance in the univariate linear model, and this may reflect low signal to noise problems well-known for PET imaging.

### Table 1. Clinical profiles and PET parameters

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Non-users ($n = 37$)</th>
<th>OC users ($n = 16$)</th>
<th>$P$-value (OC users vs. non-users)$^\dagger$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>26.9 (6.4)</td>
<td>24.5 (2.0)</td>
<td>25.8 (4.2)</td>
<td>0.71</td>
</tr>
<tr>
<td>BMI</td>
<td>23.3 (2.8)</td>
<td>23.9 (3.3)</td>
<td>22.2 (2.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Educational level</td>
<td>4.1 (1.4)</td>
<td>4.1 (1.5)</td>
<td>4.2 (1.1)</td>
<td>0.75</td>
</tr>
<tr>
<td>Individuals in relation</td>
<td>7 (26.9%)</td>
<td>3 (37.5%)</td>
<td>3 (25.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Neuroticism score</td>
<td>89.0 (19.9)</td>
<td>62.9 (19.3)</td>
<td>87.4 (21.6)</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Individuals with familial risk for MDD</strong></td>
<td>4 (13.8%)</td>
<td>0 (0.0%)</td>
<td>6 (37.5%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cohen’s PSS</td>
<td>9.4 (5.5)</td>
<td>8.6 (5.3)</td>
<td>10.1 (6.5)</td>
<td>0.66</td>
</tr>
<tr>
<td>MDI</td>
<td>4.9 (3.2)</td>
<td>4.8 (4.7)</td>
<td>4.7 (3.1)</td>
<td>0.85</td>
</tr>
<tr>
<td>SCL-92 GSI</td>
<td>0.2 (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.2 (0.2)</td>
<td>0.83</td>
</tr>
<tr>
<td>POMS-TMD</td>
<td>2.5 (18.6)</td>
<td>2.0 (18.3)</td>
<td>0.3 (12.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>P-estradiol (nmol/l)</td>
<td>0.5 (0.4)</td>
<td>0.4 (0.5)</td>
<td>0.09 (0.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P-progesterone (nmol/l)</td>
<td>3.4 (4.3)</td>
<td>1.5 (0.5)</td>
<td>1.2 (0.6)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**PET parameters**

| Individuals with HRRT scans | 26 (89.7%) | 0 (100.0%) | 13 (81.2%) | 0.36 | 53  |
| Injected dose (MBq) | 556.9 (71.1) | 502.9 (13.7) | 561.6 (105.8) | 0.91 | 53  |
| Injected SB mass/kg (µg/kg) | 0.03 (0.02) | 0.01 (0.002) | 0.02 (0.02) | 0.74 | 53  |
| Specific SB activity (GBq/µmol) | 308.4 (277.0) | 310.4 (59.9) | 265.2 (176.4) | 0.47 | 53  |

$^\dagger$ The statistical tests performed were two-sided t-test for the continuous variables, Fischer’s exact test for the categorical variables, and Gehan test for P-estradiol because of censored values.
of smaller regions. The largest difference was present in the hippocampus (−12.8% (95% CI [−21.0; −3.9]), \( P_{\text{corrected}} = 0.03 \), Fig. 3). Follow-up analyses on robustness, hormonal IUD use, and ovarian hormone associations with 5-HT4R

To overcome the issue of pooling scans from different PET scanners and having an overrepresentation of familial risk for MDD in the OC user group, other than incorporating them as covariates, we performed two separate sensitivity analyses in two restricted study groups (Table 2), (B) only HRRT scans, and (C) only HRRT scans of women with no familial risk for MDD. Both analyses supported that neither scanner type nor the distribution of familial risk for MDD was driving the observed OC effect. A supplementary analysis did not demonstrate a statistically significant association between use of hormonal IUD (\( n = 8 \)) and 5-HT4R binding relative to no use of hormonal contraception in any of the six regions; however, the analysis had clearly suboptimal power (Table S1). Further, in a supplementary analysis (Tables S3 and S4), we evaluated if the ovarian hormone status, that is, estradiol and progesterone levels, was associated with 5-HT4R binding and if any such associations depended on hormonal cycle dynamics, that is, a group interaction effect. The interaction between OC user status and the plasma estradiol level on 5-HT4R BPND was borderline significant in neocortex (\( P = 0.05 \), not corrected for multiple comparisons), pointing to a positive association between BPND and suppressed levels of plasma estradiol among OC users only. Also, the interaction was borderline significant for progesterone in ACC (\( P = 0.08 \), not corrected for multiple comparisons), such that for OC users, the BPND appeared to be negatively associated with the progesterone level.

Discussion

Here, we show that OC users have a markedly 9–12% reduction in global 5-HT4R brain binding relative to non-users. These findings are to our
knowledge the first in vivo evidence of an effect of OC use on the brain serotonin system in healthy women.

Sex steroids and serotonin signaling

Existing literature strongly supports that sex hormones, especially estradiol, affect serotonergic neuromodulation (21, 22). More specifically, both estradiol and progesterone, which directly target serotonergic neurons, may affect key features of serotonergic signaling in terms of serotonin synthesis (50, 51), degradation (51, 52), reuptake (51, 53), neuronal firing (54), and postsynaptic serotonin receptor availability on non-serotonergic neurons (55, 56). We speculate that the observed OC-associated reduction in 5-HT4R binding is a result of either (1) a direct effect of the OC’s synthetic steroids or (2) an OC-suppressed endogenous hormone state.

Knowledge on how the 5-HT4R is regulated in brain tissue is limited (57). Animal and human studies show that increased synaptic serotonergic tonus leads to a reduction in 5-HT4R (28–30, 44). Accordingly, in the context of our findings, this could imply that OC users have higher synaptic 5-HT levels. Estradiol is generally believed to support serotonergic

Fig. 2. Illustration of the global effect of OC use on 5-HT4R BP

<table>
<thead>
<tr>
<th>ROIs</th>
<th>Pct. diff. from non-users [95% CI]</th>
<th>P-value [†]</th>
<th>Pct. diff. from non-users [95% CI]</th>
<th>P-value [†]</th>
<th>Pct. diff. from non-users [95% CI]</th>
<th>P-value [†]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>−8.4 [−14.8; −1.5]</td>
<td>0.02 (0.07)</td>
<td>−7.0 [−13.8; 0.3]</td>
<td>0.06 (0.21)</td>
<td>−9.0 [−16.5; −0.9]</td>
<td>0.03 (0.11)</td>
</tr>
<tr>
<td>Caudate</td>
<td>−10.7 [−17.1; −3.9]</td>
<td>0.003 (0.01)</td>
<td>−9.0 [−15.8; −1.6]</td>
<td>0.02 (0.08)</td>
<td>−9.8 [−17.8; −0.9]</td>
<td>0.03 (0.12)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>−12.8 [−21.0; −3.9]</td>
<td>0.007 (0.03)</td>
<td>−14.7 [−21.6; −7.1]</td>
<td>0.0007 (0.01)</td>
<td>−16.5 [−24.4; −7.7]</td>
<td>0.001 (0.02)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>−6.9 [−16.1; 3.3]</td>
<td>0.17 (0.49)</td>
<td>−6.0 [−15.9; 5.1]</td>
<td>0.27 (0.69)</td>
<td>−6.4 [−17.8; 6.7]</td>
<td>0.31 (0.74)</td>
</tr>
<tr>
<td>ACC</td>
<td>−10.5 [−18.0; −2.4]</td>
<td>0.01 (0.05)</td>
<td>−9.6 [−16.6; −1.9]</td>
<td>0.02 (0.07)</td>
<td>−9.8 [−17.6; −1.4]</td>
<td>0.02 (0.09)</td>
</tr>
<tr>
<td>Neocortex</td>
<td>−10.9 [−18.2; −3.0]</td>
<td>0.009 (0.04)</td>
<td>−9.7 [−17.2; −1.6]</td>
<td>0.02 (0.09)</td>
<td>−10.3 [−18.8; −0.9]</td>
<td>0.03 (0.12)</td>
</tr>
</tbody>
</table>

(A) Main analysis with all scans; (B) only High-Resolution Research Tomograph (HRRT) PET scans; (C) only HRRT PET scans of the women without any familial risk for major depressive disorder.

†P-values after adjustment for six comparisons using a single-step Dunnett’s procedure. BPND; non-displaceable binding potentials, ROIs; regions of interest, ACC; anterior cingulate cortex.
Therefore, if an increased 5-HT tonus should explain the lower levels of 5-HT4R in OC users, it may be related to synthetic steroid exposure from OCs. However, since estrogen mainly target serotonergic neurons via ERβ activation (59–62), ethinyl estradiol, most likely, does not directly stimulate serotonergic neurotransmission since it primarily stimulates ERα (63). This may even compromise serotonergic neurotransmission as ERα activation increases the 5-HT1A auto-receptor expression, which can inhibit 5-HT-firing from dorsal raphe nucleus (64, 65). Taken together, this tends to argue against increased 5HT levels induced by ethinyl estradiol from OCs. On the other hand, the progestin from OCs could interfere with the serotonergic neurotransmission, but this is less well understood. Alternative to an increased serotonergic tonus, the lower 5-HT4R binding may be caused by a hormone-dependent reduction of gene expression of the 5-HT4 receptor (66) because of OC-induced suppression of endogenous estradiol (Table 1). In support of this explanation, estradiol increases the expression levels of the 5-HT4R in the rat anterior pituitary cells (67).

As expected, the OC users had suppressed estradiol and progesterone levels compared to non-users (69). It is well established that OC users have a non-dynamic hormone status compared to non-users (18). We speculate that a dynamic vs. non-dynamic hormone status may be critical for the regulation and adaptive capacity of cerebral 5-HT4R. Similarly, this might be why hormonal IUD use shows no effect on 5-HT4R binding. However, because of low statistical power on the supplementary hormonal IUD analysis, we cannot conclude on this. Future work on 5-HT4R BPND and hormonal IUD use is wanted since IUD also has been associated with the development of depression (6). Nevertheless, confounding by indication can also play a role in the earlier observed association between hormonal IUD use and depression-related phenomena.

We observed a trend toward an association of estradiol and progesterone with the binding in neocortex and ACC in the OC user group but not in the non-user group (Tables S3 and S4), that is, a positive association for estradiol and negative association for progesterone on the 5-HT4R binding. Larger independent datasets are needed to corroborate these findings and determine if the effect can be attributed to blunted levels of specific hormones or related metabolites. For example, the neuroactive progesterone metabolite, allopregnanolone, which is reduced in OC users (69), is of particular interest in that context. So far, no human data exist to clarify whether allopregnanolone is associated with 5-HT4R binding; however, allopregnanolone appears to be negatively associated with cerebral serotonin transporter binding in naturally cycling women (70).
alone we are not able to conclude if the lower 5-HT4R binding level has protective or harmful effects in the context of depression. Previous research on the association between OC use and depression is inconsistent (11). One of the reasons might be that the mood- and affect-related effects of OC use are subclinical for most women, which is supported by studies showing that OC users appear less responsive to emotional stimuli including positive stimuli (71, 72).

Other hormonal transitions may also be linked to depressive states: For example, earlier data from our group link the emergence of depressive symptoms with sex hormone manipulations and changes in serotonin transporter binding, which tend to compromise serotonin signaling (73). Further, evidence converges to support the notion that hormonal transitions may cause depressive symptoms in hormone-sensitive individuals: (i) Estradiol fluctuations around menopausal transition are associated with first-time onset of MDD (74) and appears to be preventable by hormonal replacement (75), (ii) hormone manipulations trigger depressive symptoms in women with a history of postpartum depression (76), and (iii) 'estrogen sensitivity' at the level of gene transcription and DNA methylation also appears to increase risk for postpartum depression (77, 78). Intriguingly, we do not yet know if depressive responses to OC use may be a marker of such hormone sensitivity.

Another line of evidence directly links 5-HT4Rs to MDD (79); two polymorphisms in the splice variant region of the 5-HT4R gene have been associated to MDD (79), and hippocampal 5-HT4R binding is reduced in the Flinders Sensitive Line rat model of depression (29). 5-HT4Rs appear to play a role in familial risk for MDD (43). As 5-HT4R activation has widespread effects on serotonergic neurotransmission, that is, stimulates the serotonergic neuronal firing from the dorsal raphe nuclei to the rest of the brain (80), OC-induced changes in 5-HT4R agonism capacity may distort the serotonin system and increase risk of depression and/or play a role in antidepressant treatment. Notably, rodent data support fast acting antidepressant-like effect of 5-HT4 partial agonists (26), and that antidepressant-like effects of selective serotonin reuptake inhibitor (SSRI) is dependent on 5-HT4R activation (81).

SSRIs are known to reduce 5-HT4R binding as shown in animal (29, 30) and human (28) studies. In healthy men, three weeks of SSRI treatment reduces the 5-HT4 BPND by 5% (28), and this most likely represents as an adaptive response to the heightened serotonergic tone. The observed effect size of OC use on the 5-HT4R is comparatively large, that is, approximately twice as big. Also, it is considerably larger than the test–retest difference for [11C]SB207145 ligand, which is 4–7% depending on the region (25). This questions whether concomitant use of OCs in depressed individuals could affect SSRI treatment response. As far as we know surprisingly, only the STAR*D study has addressed this question, which followed 896 depressed premenopausal women, 226 of whom used hormonal contraceptives (82). The authors found a trend toward better remission rate among the hormonal contraceptive users. This may argue for a protective effect of the reduced 5-HT4R level in OC users, but alternatively it also reconciles with the notion that if OC use contributes to the depressed state by suppressing 5-HT4R capacity, then SSRIs may work particularly well in OC users by increasing serotonergic tone and thus support 5-HT4R agonism.

Interestingly and yet unstudied, withdrawal of OCs may itself have an antidepressant effect. Further studies are warranted to clarify whether (i) concomitant use of OCs affect SSRI treatment response and (ii) whether women who develop a depressive episode while taking an OC (even in cases where OCs were not the trigger of MDD) may benefit from withdrawal of OCs either alone or in combination with SSRI treatment. It is important to emphasize that not all women using OCs develop depressive episodes, and correspondingly, none of the OC using women in this study were depressed. Nevertheless, an OC-induced decrease in 5-HT4R agonism capacity may trigger depressive episodes in some women, perhaps a particular subgroup of hormone-sensitive women. How lower 5-HT4R BPND otherwise may affect healthy women using OCs remains elusive but can include effects on cognition. Intriguingly, we do see the largest effect size seen in the hippocampus, which is key in memory functions. OC use may affect memory performance (83), where 5-HT4Rs also appear related (84), as shown repeatedly in humans, in particular for hippocampal 5-HT4R binding (85, 86). This might be related to the role of 5-HT4R activation in hippocampal neurogenesis (87). Several studies have investigated the effect of OC use on hippocampal volume, but results are inconsistent (17, 88, 89). Future studies are needed to address neurodegenerative long-term effects of OC use, for example, Alzheimer’s disease, where 5-HT4Rs may play a protective role (90, 91), as well as to what extent withdrawal from OCs rescues 5-HT4R brain binding.

Taken together, OC users have 9–12% lower 5-HT4R BPND. This adds new insight into a
plausible mechanism by which OC use may increase the risk of developing depressive episodes. If confirmed in future longitudinal studies and if translated to other hormonal transitions across reproductive age, a history of OC-induced depressive symptoms may serve as a key marker for healthcare professionals to guide psychoeducation or personalize prevention and treatment strategies.

Methodological considerations

Some limitations should be considered when interpreting the results of this study. First, as no information was collected about pill-free days or experienced OC-related mood deterioration, we cannot relate these to potential patterns of 5-HT4R binding. Also because of limited power, no firm conclusion could be drawn from the current dataset on a potential moderate to small effect of menstrual phase on 5-HT4R binding; however, we saw no effect of menstrual cycle phase per se in our data (8 in luteal phase vs. 14 in follicular phase, $P > 0.2$). Likewise, existing data on menstrual phase effects on other serotonergic markers; that is, the serotonin 1A receptor and the serotonin transporter do not support menstrual phase to affect serotonergic signaling in a detectable manner in the absence of psychopathology (92). Further, we were underpowered to take potential effects of smoking on 5-HT4R binding into account in our analyses, since we only had 3 smokers. When ignoring these three in the analyses, it did not affect the results. Second, we cannot rule out a possible ‘survival effect’ among the OC users, such that those sensitive to OC-depressogenic effects converted to non-users before inclusion or those who developed depression on OCs were not included as healthy controls. Therefore, our sample might be biased, so if more ‘vulnerable’ women had been included, a larger effect may have appeared. Third, three different Siemens MAGNETOM MRI scanners were used. The effect of MR scanner on 5-HT4R $B_{PD_{ND}}$ is expected to be low and thus not expected to drive our results, which was confirmed by sustained effects seen in analyses restricted to Prisma scans only ($n = 41$, results not presented). Fourth, because of a limited sample size, the derived effect estimates of the associations between ovarian hormone steroids and 5-HT4R binding are estimated with large uncertainty.

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Declaration of interest

VGF and GMK declare to have received honorarium for advisory meetings at Sage Therapeutics and VGF for a symposium lecture at Lundbeck Pharma A/S. All others authors declare no potential conflict of interest.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/acps.13211. [Correction added on 14 October 2020, after first online publication: Peer review history statement has been added.]

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request and from the Cimbi database of molecular brain PET in healthy humans (1). Access can be requested through the procedures outlined here: https://cimbi.dk/index.php/documents/category/3-cimbi-database.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:

Table S1 No significant association between hormonal IUD use and serotonin 4 receptor BP\textsubscript{ND}.
Table S2 Overview of the specific type of combined oral contraceptives used.
Table S3 Group BY plasma estradiol interaction effects on serotonin 4 receptor BP\textsubscript{ND} at different plasma estradiol imputations.
Table S4 Group BY plasma progesterone interaction effects on serotonin 4 receptor BP\textsubscript{ND}.