Estradiol modulates changes in effective connectivity in emotion regulation networks

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ABSTRACT

Hormonal changes in ovarian hormones like estradiol (E2) during the menstrual cycle affect emotional processes, including emotion recognition, memory, and regulation. So far, the neural underpinnings of the effect of E2 on emotional experience have been investigated using task-based functional magnetic resonance imaging (fMRI) and functional connectivity. In the present study, we examined whether the intrinsic network dynamics at rest (i.e., directed effective connectivity) related to emotion regulation are (1) modulated by E2 levels and (2) linked to behavioral emotion regulation ability. Hence, 29 naturally cycling women participated in two resting-state fMRI scans in their early follicular phase after being administered a placebo or an E2 valerate, respectively. Emotion regulation ability was assessed using a standard emotion regulation task in which participants were asked to down-regulate their emotions in response to negative images. The regions of two functionally predefined neural networks related to emotional down-regulation and reactivity were used to investigate effective connectivity at rest using spectral dynamic causal modelling. We found that E2, compared to placebo, resulted in changes in effective connectivity in both networks. In the regulation network, prefrontal regions showed distinct connectivity in the E2 compared to the placebo condition, while mixed results evolved in the emotional reactivity network. Stepwise regressions revealed that in the E2 condition a connection from the parietal to the prefrontal cortex predicted regulation ability. Our results demonstrate that E2 levels influence effective connectivity in networks underlying emotion regulation and emotional reactivity. Thus, E2 and its potential modification via hormonal administration may play a supporting role in the treatment of mental disorders that show a dysregulation of emotions.

1. Introduction

Sex and gender are influential factors in our mental health. Women are differently affected by mental disorders than men not only in terms of prevalence rates but also with regard to symptomatology, severity, course of illness, help-seeking behavior, and treatment efficacy (Mauvais-Jarvis et al., 2020; Riecher-Rossler, 2017). Importantly, the onset and potential relapse concerning these disorders are often related to reproductive events like puberty, pregnancy, the postpartum period and the transition to menopause, as well as the physiological cyclicity of the menstrual cycle (Soares and Zitek, 2008), which are all characterized by changes in sex hormone levels. A naturalistic model to

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investigate the impact of endogenous hormonal fluctuations on the brain and behavior is the female menstrual cycle. It is characterized by low estradiol (E2) and progesterone during the early follicular phase, high E2 but low progesterone during the peri-ovulatory phase, peak progesterone and medium E2 levels in the mid-luteal phase, and decreasing levels of both hormones during the premenstrual/late luteal phase. A growing body of literature indicates that the menstrual cycle phase, as well as related hormonal changes, impact several cognitive and emotional processes, including emotional experience, emotion recognition and emotional memory (Dernitl et al., 2008a; Gamsakhurdashvili et al., 2021; Kimig et al., 2021; Sundström-Poromaa, 2018). Consequently, differences in brain activation during the processing of emotional stimuli have been linked to the menstrual cycle phase (Dernitl et al., 2008b; Dubol et al., 2021; Jacobs et al., 2015).

Notably, there are also first hints that emotion regulation - the ability to change our emotions in timing, type, experience and expression (Gross, 1998) - is affected by the menstrual cycle phase. The most widely investigated emotion regulation strategy is reappraisal that involves reframing the meaning of a situation to alter its emotional impact (Gross, 1998). A handful of studies have investigated emotion regulation across the menstrual cycle using self-report measures and behavioral tests, indicating that females who have greater emotion regulation difficulties also report less control over anxiety-related events, especially during the late luteal phase (Manikandan et al., 2016). Furthermore, the menstrual cycle phase may influence the regulation strategy’s effectiveness. For example, suppression of disgust is as effective as reappraisal during the entire follicular phase, while a similar effect of suppression was not observed during the entire luteal phase (Olatunji et al., 2020). Wu et al. (2014) demonstrated that while early follicular females reported using reappraisal more often, subjective reports of sadness did not differ across the menstrual cycle. Further, the effects of reappraisal on skin conductance responses were smaller in the early follicular than in the mid-luteal phase. According to these results, reappraisal seems less efficient during the early follicular phase than during the mid-luteal phase.

Regarding neural activation, Lusk et al. (2017) investigated the effect of the menstrual cycle phase on cortical processing during emotion regulation using reappraisal and suppression, i.e., the conscious inhibition of emotional expressive behavior while emotionally aroused. The study revealed greater amplitudes of the event-related potentials N1 and P1, which represent early negative (N) and positive (P) potentials ca. 50–150 msec after stimulus onset, to unpleasant stimuli in mid-luteal females relative to early follicular females and males, indicating an early attentional bias in occipital (P1) and frontocentral (N1) regions. In the suppression condition, mid-luteal females showed a significantly higher N2 amplitude than males, a negative potential occurring around 200–350 msec after stimulus onset, indicating higher cortical processing in frontocentral areas and greater difficulties in suppressing responses to negative emotional stimuli. This was paralleled by greater distress ratings in mid-luteal females compared to early follicular females (trend) and males (significant) in the suppression condition. Further, progesterone correlated with the N2 amplitude during suppression and not reappraisal in mid-luteal females.

In a recent randomized controlled study, Rehbein et al. (2021) administered E2 valerate or placebo to females in the early follicular phase, pushing their E2 levels to peri-ovulatory concentrations within 24 hrs and then asked them to perform an emotion regulation task during neuroimaging. While applying reappraisal to negative emotional stimuli, changes in activity in the inferior (IFG) and middle frontal gyrus (MFG) were higher in the placebo than in the E2 condition, while no significant behavioral difference emerged. Therefore, E2 may facilitate reappraisal processing by requiring less inferior and middle frontal activity for comparable behavioral outcomes. This fits nicely with findings from an earlier study, where a significantly stronger reduction of physical arousal in a fear extinction task after cognitive restructuring training was observed in females during the peri-ovulatory phase (high E2) compared to females during the early follicular phase (low E2) (Graham et al., 2017). The suggested facilitation effect of E2 on emotion regulation and neural activation seems already apparent in adolescent girls, as higher E2 levels predicted enhanced activity in the dorsolateral prefrontal cortex (dlPFC) during the reappraisal of negative emotions (Chung et al., 2019). Taken together, these studies clearly highlight an association between the menstrual cycle phase, E2 and brain activity related to emotional reactivity and emotion regulation. However, so far, only a few studies have investigated whether functional or effective connectivity underlying emotional reactivity and regulation are also affected by the menstrual cycle phase and E2.

In terms of functional connectivity, previous studies showed a significant influence of the menstrual cycle phase on seed-based - mostly amygdala, hippocampus, and striatum - as well as network-based analyses, most commonly on connectivity within and between the default mode network (DMN), salience network (SN) and executive control network (ECN) (Cenjaers et al., 2023; Dubol et al., 2021; Hidalgo-Lopez et al., 2020; Lisofsky et al., 2015; Weis et al., 2019). Moreover, within the DMN, an increase in intrinsic resting-state connectivity has been associated with E2 during the peri-ovulatory phase (De Bondt et al., 2015; Hidalgo-Lopez et al., 2021; Mueller et al., 2021; Pritsch et al., 2020) while decreases were related to progesterone during the mid-luteal phase (De Filippi et al., 2021; Hidalgo-Lopez et al., 2021). Regarding effective connectivity, Hidalgo-Lopez et al. (2021) demonstrated an anterior-posterior modulation due to the menstrual cycle phase in the DMN and changes in insula connectivity within the ECN due to fluctuating E2. Hence, these results support E2’s suggested facilitation role and add the direction in which E2 impacts intrinsic connectivity.

Previous research indicates that the menstrual cycle phase and E2 influence behavior, brain activity and connectivity related to emotion regulation. However, whether the intrinsic network architecture at rest is also affected by E2 and linked to the ability to regulate one’s emotions remains unknown. Therefore, the present study examines the impact of E2 administration on effective connectivity at rest within two networks underlying emotional reactivity and regulation (Morawetz et al., 2022) using spectral dynamic causal modelling (sDCM; Friston et al., 2014). SpDCM allows us to investigate the complexity within and between network relationships and the causal influence one node exerts over another (Hidalgo-Lopez et al., 2021).

The aim of the current study was two-fold: (1) For the first time, we examined differences in intrinsic neural network dynamics before and after E2 administration within the same sample, and (2) we explored the relationship of effective connectivity within two predefined neural networks (one network involved in emotional reactivity and one related to emotion regulation) and the ability to down-regulate emotions using reappraisal. Since there is limited primary research regarding effective connectivity underlying emotion regulation (Underwood et al., 2021) and the link between effective connectivity and ovarian hormones (Hidalgo-Lopez et al., 2021), we put forth the following exploratory hypotheses: Firstly, we hypothesized a difference in resting-state effective connectivity (rs-EC) between the E2 and the placebo condition in two predefined neural networks. Secondly, some connections that differ in their rs-EC between the conditions should predict the behavioral emotion regulation ability assessed in a separate emotion regulation task. To do so, we analyzed resting-state data as part of a larger project related to the effects of E2 administration on brain function. Effects of E2 administration on emotion regulation using task-based fMRI have been previously published (Rehbein et al., 2021).

2. Methods

2.1. Sample

Thirty-two naturally cycling females participated in the study (M_{age}=24.1±3.1 years). Prior menstrual cycle tracking confirmed a
regular cycle duration of 26–32 days. Magnetic resonance imaging (MRI) contraindications (e.g., metal implants), present or past mental, neurological or endocrine disorders, use of hormonal contraceptives during the last six months, any other medication intake, or past and current pregnancies were exclusion criteria. Participants were recruited via a student e-mail provider from the University of Tübingen. The Ethics Committee of the Medical Faculty of the University of Tübingen approved the study (754/2017/BO1). All participants signed an informed consent and data protection agreement. We included data from 29 females in the functional magnetic resonance imaging (fMRI) and behavioral analyses, excluding three due to an incomplete dataset, excessive head movement and oral contraceptive intake. A detailed sample description, including E2 levels, questionnaire data and emotional state ratings, is presented in Table 1. Participants had a mean body-mass-index (BMI) of 23.48 (SD=3.93). Self-reported use of emotion regulation strategies was measured via the German version of the Emotion Regulation Questionnaire (ERQ; Abler and Kessler, 2009; Gross and John, 2003).

The number of participants for this study was chosen based on a power calculation to detect effects of E2 administration on task-based parameters presented in Rebbein et al. (2021). Moreover, it was shown that a sample size of n=20 already provides reliable DCM predictions (Goulden et al., 2012).

2.2. Procedure

The experimental procedure is illustrated in Fig. 1. After an initial screening interview, where BMI, diet, menstrual cycle length and onset, and ERQ were assessed, participants were invited to an appointment 2–5 days after their menstruation onset, i.e., during the early follicular phase of the menstrual cycle. We based our administration regime on findings of previous studies that also administered 6 mg of E2 valerate (Bayer et al., 2018; Kuhl et al., 2005) and indicated that this dosage is well tolerated by participants, has no severe side effects and effectively increases E2 levels. Of note, the half-life of E2 valerate is estimated to be around 12–14 hrs, and as a consequence, administering a second dose after approximately 12 hours can enhance peak levels further (cf. Bayer et al., 2018). Before the first fMRI scan, on two consecutive days, they orally took either a placebo sugar pill or 6 mg of an E2 valerate (Progynova®) so that in the E2 condition, E2 measures matched pre-ovulatory levels over multiple hours. After 2–3 months, again in the early follicular phase, they were assigned to the other drug condition to take the pills on two consecutive days before the second fMRI appointment. Medications were administered in a double-blind and counter-balanced order. On the first day, women were issued the first pill and completed the Beck Depression Inventory (BDI-2; Kühner et al., 2007) to control for possible effects. Approximately 14–18 hrs after the first pill intake, they received the second pill. Approx. six hours after the second pill intake (when E2 levels reach its peak), participants underwent a rs-fMRI scan. Subsequently, they performed an emotion regulation task inside the scanner. Participants were exposed to aversive pictures and instructed to either down-regulate (i.e., decrease) their upcoming negative emotions (decrease condition) or experience their emotional responses without regulating them (maintain condition). Positive and negative affect (PANAS; Krohne et al., 1996) and state anxiety (STAI; Spielberger et al., 2001) were assessed before and after the emotion regulation paradigm. To determine hormonal levels, blood samples were drawn by medical staff before the first pill intake as well as right before the fMRI scans (thus when E2 levels reach its peak). Side effects of drug intake and menstruation onset were to be declared by the participants.

2.3. Emotion regulation task

A detailed description of the task can also be found in Rebbein et al. (2021). Briefly, stimuli for the decrease task were pictures from the International Affective Picture System (IAPS; Lang and Bradley, 2007). The paradigm was adapted from Morawetz et al. (2016). The pictures were displayed using the Presentation® Software Version 20 (Neuro-behavioral Systems) with a size of 800×600 pixels, a visual angle of 32°×24° and on a black background. In two runs, both including 12 highly aversive pictures (ratings from the IAPS picture database: M_valence=−2.92, SD_valence=1.62; M_around=5.18, SD_around=2.23), participants were instructed to either maintain upcoming emotions without changing them or to decrease their emotional response. Decreasing emotions was done by applying reappraisal, i.e., by perspective-taking, distancing or reinterpreting the meaning of the picture. In an event-related design, the pictures were shown twice for 8 sec, once for each instruction. The instruction and regulation phase was separated by a jittered fixation cross (2–6 sec). After the regulation phase, participants indicated their current emotional state on a continuous visual analogue rating scale from very negative (−200) to very positive (+200).

2.4. fMRI data acquisition

FMRI data were acquired at a 3 T Siemens PRISMA scanner at the University Hospital Tübingen. The fMRI sequence consisted of a standard Echo Planar Imaging (EPI) protocol (32 interleaved slices, TR=2000ms, TE=32ms, voxel size=3.4×3.4×3.4 mm, flip angle=76°, transversal orientation, anterior-posterior commissure orientation, 64-channel head coil, field of view=220×220 mm). In addition, an anatomical image was acquired (MPRAGE; TR=2300ms, TE=4.16ms, voxel size=1×1×1 mm, flip angle=9°, distacing factor 50%, GRAPPA acceleration factor, sagittal orientation, field of view=256×256 mm). Threehundred volumes were acquired during one

<table>
<thead>
<tr>
<th>E2 levels</th>
<th>Placebo condition</th>
<th>t-value/Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 Day 1 (T1/4)</td>
<td>197.08 (92.87)</td>
<td>186.69 (76.76)</td>
<td>Z(26) = −0.28</td>
</tr>
<tr>
<td>E2 Day 2 (T2/15)</td>
<td>572.40 (194.66)</td>
<td>206.52 (99.66)</td>
<td>Z(22) = −4.11</td>
</tr>
<tr>
<td>Questionnaires</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI-2</td>
<td>6.48 (5.58)</td>
<td>6.41 (5.19)</td>
<td>Z(27) = −0.81</td>
</tr>
<tr>
<td>Positive Affect</td>
<td>32.24 (5.59)</td>
<td>32.22 (3.61)</td>
<td>Z(27) = −0.57</td>
</tr>
<tr>
<td>Negative Affect</td>
<td>19.72 (3.37)</td>
<td>20.96 (4.91)</td>
<td>Z(28) = −1.98</td>
</tr>
<tr>
<td>STAI</td>
<td>37.59 (6.15)</td>
<td>36.61 (7.82)</td>
<td>Z(28) = −0.13</td>
</tr>
<tr>
<td>Emotional state ratings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>−30.46 (37.09)</td>
<td>−27.05 (32.95)</td>
<td>Z(28) = −.97</td>
</tr>
<tr>
<td>Maintain</td>
<td>−57.17 (30.38)</td>
<td>−62.13 (33.74)</td>
<td>t(28) = 1.14</td>
</tr>
<tr>
<td>Emotional regulation ability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Decrease – Maintain)</td>
<td>26.72 (44.82)</td>
<td>35.08 (37.45)</td>
<td>Z(28) = −1.48</td>
</tr>
</tbody>
</table>

Notes. E2 = estradiol, BDI-2 = Beck Depression Inventory 2, STAI = State-Trait Anxiety Inventory; Mean values are displayed, and standard deviations are shown in brackets.
resting-state run. Participants were instructed to keep their eyes closed, not fall asleep, and not think about anything in particular.

2.5. Hormonal analysis

Hormonal levels of E2, progesterone and testosterone were analyzed at the central laboratory of the University Hospital Tübingen. 7.5 ml of blood was drawn in serum tubes and analyzed using enzyme-linked immunosassays (ELISA). Sensitivity and range of measurement for E2, progesterone, and testosterone were: E2: 43.60–11,010 pmol/l; progesterone: 0.67–190.80 nmol/l; testosterone: 0.24–52.05 nmol/l.

2.6. Data analyses

2.6.1. Hormone data

All hormonal and behavioral data analyses were performed using IBM SPSS Statistics, Version 27. E2 levels were analyzed using the Wilcoxon signed rank test since normal distribution was violated. Hormonal data have been analyzed and reported previously by Rehbein et al. (2021).

2.6.2. Analyses of emotional state ratings and questionnaires

Emotion regulation ability was calculated by subtracting the mean emotional state rating of the maintain condition from the decrease condition. We refer to the mean emotional state ratings of the maintain condition as emotional reactivity in response to negative images. Due to variables not being normally distributed, a Wilcoxon signed-rank test was used to analyze differences between drug conditions in emotion regulation ability. A t-test for paired samples was performed to test for differences between drug conditions in emotional reactivity. BDI-2, PANAS and STAI were analyzed using the Wilcoxon signed-rank test. Correlation analyses reporting associations between hormone levels and emotional state ratings and their interpretation have been reported in Rehbein et al. (2021).

2.6.3. fMRI data analyses

Preprocessing. Rs-fMRI scans were preprocessed using SPM12 (Statistical Parametric Mapping, Wellcome Centre for Human Neuroimaging, London, UK) in Matlab R2018b (MathWorks, Natick, MA, USA). The first five slices of the functional data were removed as dummies. The preprocessing steps consisted of slice-time correction, realignment to the first volume, coregistration to each participant’s T1-weighted scan, spatial normalization to the standard EPI template (Montreal Neurological Institute, MNI), and smoothing with an isotropic Gaussian kernel (6 mm full-width at half-maximum). None of the participants had head motion exceeding 2 mm.

Region-of-interest (ROI) selection and time series extraction. ROIs for the effective connectivity analysis were taken from a recent meta-analysis on emotion regulation (Morawetz et al., 2022b). The predefined ROIs are involved in increasing and decreasing emotions using reappraisal. Two neural networks were used for the analysis: one network associated with emotion regulation (regulation network) and the second one related to emotional reactivity (reactivity network). The predefined ROIs are illustrated in Fig. 2.

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The regulation network consisted of the following frontal, temporal and parietal ROIs: left inferior frontal gyrus (LIFG, MNI: x = -46, y = 28, z = -8), right inferior frontal gyrus (RIFG, MNI: x = 50, y = 30, z = -8), right middle frontal gyrus (RMFG, MNI: x = 42, y = 24, z = 40), left mediofrontal gyrus (LMeFG, MNI: x = -4, y = 12, z = -2), left middle temporal gyrus (LMTG, MNI: x = -60, y = -38, z = -2), left superior temporal gyrus (LSTG, MNI: x = -42, y = -56, z = 24) and right supramarginal gyrus (RSMG, MNI: x = 58, y = -54, z = 38).

The reactivity network originally included ten temporal, parietal and limbic ROIs. In this study, we used the following eight ROIs (excluding the two thalamic ROIs due to susceptibility artefacts in the current data set): left inferior temporal gyrus (LITG, MNI: x = -48, y = -74, z = 2), right inferior temporal gyrus (RIFG, MNI: x = 50, y = -64, z = -4), left inferior parietal lobe (LIPr, MNI: x = -58, y = -24, z = 32), right inferior parietal lobe (RIPr, MNI: x = -62, y = -22, z = 32), left precuneus (LPrec, MNI: x = -26, y = -50, z = 56), right precuneus (RPrec, MNI: x = 26, y = -56, z = 54),
left amygdala (LAmy, MNI: x=-22, y=-6, z=-16), and right amygdala (RAmy, MNI: x=24, y=-4, z=-16).

The preprocessed data were used to establish the residuals of a General Linear Model (GLM). Blood-oxygen-level-dependent (BOLD) time series were extracted for all predefined ROIs. Six head motion parameters and white matter/cerebrospinal fluid signals were added to the GLM as nuisance regressors. To extract the time series from each ROI, the peak coordinates were the center of a 6 mm sphere using the principal eigenvector of all included voxels. The high-pass filter was set at 128 sec.

Spectral dynamic causal modelling. To examine the effective connectivity between ROIs within the networks, spectral dynamic causal modelling (spDCM) was performed using DCM12 as implemented in SPM12. GLMs and classical DCMs are used to model predicted data onto the GLM, which is then used to model the residuals. This process is repeated until the model fitted the data best according to a used as dependent variables. The first regression analysis was performed to predict emotion regulation ability from the significant effective connections at rest from the regulation network (see Table 2). A second model was performed to predict emotional reactivity again using the significant effective connections at rest from the activity network (see Table 2). For both regression analyses, change in E2, progesterone and testosterone (from Day 1 to Day 2) were included as further predictors, whereas age, menstrual cycle length, menstrual cycle day, BMI and diet were used as control variables.

3. Results

3.1. Hormone data

As previously reported by Rehbein et al. (2021), Wilcoxon signed-rank tests revealed significantly higher E2 levels in the E2 condition than the placebo condition on Day 2 (T3/T6) but not on Day 1 (T1/T4) (see Table 1). Additionally, results showed a significant increase between Day 1 and Day 2 in the E2 condition, $Z=-4.2, p<.001$, and in the placebo condition, $Z=-2.27, p=.022$. The increase (difference between Day 1 and 2) was significantly steeper in the E2 condition than in the placebo condition, $Z=-4.02, p<.001$.

3.2. Behavioral data & questionnaires

There was no difference between the E2 and placebo condition in the behavioral emotion regulation ability and emotional reactivity during the decrease task, as already reported by Rehbein et al. (2021). No group difference was found between the conditions in scores of the BDI-2 as measured on Day 1. Day 2 revealed no group difference in positive affect, negative affect and state anxiety. The results are summarized in Table 1.

3.3. fMRI results

For the regulation network, the first-level analysis revealed a high model convergence. The variance predicted by each participant’s reduced model was, on average, $M=89.71$ (SD=2.27, range=83.01–95.13). The reactivity network showed a model convergence on average with a variance of $M=89.33$ (SD=3.22, range=78.83–95.96). Second-level analyses revealed differential connectivity patterns for both neural networks between the two conditions (E2 vs. placebo condition), as shown in Fig. 3. Positive values are indicated in green and represent increased connectivity after E2 administration compared to placebo, while negative values (indicated in orange) represent decreased connectivity after E2 administration compared to placebo. Table 2 shows the results in detail and states which connections are excitatory or inhibitory.

We used Bayesian model comparison to analyze possible models. Models assumed that a different combination of connectivity parameters can characterize all participants. One or more connections were removed for each reduced form of the full model. The parameters of reduced PEB models were derived analytically using Bayesian Model Reduction (BMR). The best models were averaged and weighted by their model evidence using Bayesian Model Averaging (BMA). Drug condition was modelled as the main regressor of interest to determine the differences in effective connectivity between the conditions (E2 vs. placebo) relative to the mean. The connectivity between ROIs is indicated in Hertz (Hz), where positive values represent an excitatory connection and negative values represent an inhibitory connection.
Regulation network. When determining the effects of E2 on effective connectivity in the regulation network, more inhibitory and excitatory interconnectivity within prefrontal regions after E2 administration compared to placebo was revealed (e.g., from LIFG to RMFG, LIFG to LMeFG, RIFG to LIFG, RIFG to RMFG, RMFG to RIFG) (Fig. 3 left, Table 2). Only one connection within the prefrontal regions, from the RIFG to the RMFG, showed less inhibition in the E2 compared to the placebo condition. Thus, 80% of the modulated connections within the prefrontal regions were more connected after E2 administration. Additionally, the connection from the LMeFG to the LMTG, as well as the bottom-up connection from the LSTG to the RIFG, were more inhibitory in the E2 compared to the placebo condition. Two connections from temporal and parietal regions to prefrontal regions, more specifically from the RSMG to the LIFG (inhibitory) and from the LMTG to the LMeFG (excitatory), showed less connectivity in the E2 condition. Overall, the majority (67%) of the effective connections in the regulation network that showed a difference between the conditions were inhibitory, and 33% were excitatory, with the most prominent changes in the prefrontal regions. Further, every connection within the regulation network that was modulated by E2 was either within, from or to prefrontal regions.

Reactivity network. In the reactivity network, a mixed pattern of results evolved with a number of inhibitory and excitatory connections that were modulated by E2 administration (Fig. 3 right, Table 2). More connectivity in the E2 than in the placebo condition was revealed for several connections, including RPrec to bilateral amygdalae and RAmy to RPrec (inhibitory), RAmy to Lamy and RAmy to LITG (excitatory), as well as the LITG to the RIPL (inhibitory). Connections from parietal regions, especially RIPL, to temporal and parietal regions were mostly less connected after E2 administration compared to the placebo condition (RIPL to LITG, RIPL to RITG, LPrec to LITG and LPrec to RITG). Only one parietal-temporal connection was more connected in the E2
within the same sample. The findings revealed that top-down emotion-

regulatory and bottom-up emotion-generating networks are modulated by E2 administration by increasing the connectivity between regions. Moreover, we show that some of the connections that differed between drug conditions were predictive of regulation ability and emotional reactivity in a behavioral emotion regulation task.

Inhibitory connectivity during rest within the prefrontal cortex (PFC) – including bilateral ventrolateral PFC (left and right IFG), right dorsolateral PFC (right MFG) and dorsomedial PFC (left MeFG) – was increased in the E2 condition compared to the placebo condition. This finding is in line with the task-based activation results of the same sample. Rehbein et al. (2021) reported that activity in the left ventrolateral PFC decreased during the task after E2 administration compared to the placebo condition. Together, these findings indicate that E2 administration might change the intrinsic dynamics in prefrontal cortex regions by supporting an increase in inhibitory effects, which further decrease activity during the active down-regulation in the same regions and, thus, result in less neuronal effort. This idea is supported by animal and human research, indicating that E2 improves prefrontal-dependent functions (Luine, 2014). In general, the lateral PFC regions are implicated in maintaining the regulation goal, directing attention to the emotional stimulus, and actively reinterpreting the stimulus (Morawetz et al., 2020). The observed inhibitory interconnectivity within the PFC in this study extends previous findings of task-based effective connectivity during emotion regulation (Morawetz et al., 2016). Latter study reported bidirectional changes in connectivity between ventrolateral PFC and dorsolateral PFC, indicating a potential feedback mechanism that supports the selection process of goal-appropriate reappraisals. Here, we also determined one reciprocal frontal connection between the right dorsolateral PFC and ventrolateral PFC. This enhanced recruitment of the right lateral PFC is in accordance with previous findings reporting increased integration between the default mode network (DMN) and executive control network (ECN) - of which the lateral PFC is part – during the early follicular phase that is characterized by low levels of E2 (Hidalgo-Lopez et al., 2021). Thus, the increased inhibitory state at rest might represent a preappratory state needed to ensure effective emotion regulation that is boosted by E2 and reflected in enhanced interconnectivity between key cognitive control regions. In addition, regions of the PFC are, besides other areas of the brain like the amygdala and hippocampus, dense in estrogen receptors (Almey et al., 2015; Barth et al., 2015) and animal research points to E2-dependent synaptogenesis.

### Table 3

<table>
<thead>
<tr>
<th>Emotion regulation</th>
<th>Predictor</th>
<th>β</th>
<th>t</th>
<th>p</th>
<th>R</th>
<th>R²</th>
<th>ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>RSMG → LIFG</td>
<td>.469</td>
<td>2.317</td>
<td>.032</td>
<td>.469</td>
<td>.220</td>
<td>.179</td>
</tr>
<tr>
<td>Placebo</td>
<td>RSMG → LIFG</td>
<td>.455</td>
<td>2.285</td>
<td>.033</td>
<td>.455</td>
<td>.207</td>
<td>.167</td>
</tr>
<tr>
<td>Emotional reactivity</td>
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<td></td>
<td></td>
<td></td>
<td>.832</td>
<td>.692</td>
<td>.614</td>
</tr>
<tr>
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<td>3.894</td>
<td>.001</td>
<td>.617</td>
<td>.389</td>
<td>.687</td>
</tr>
<tr>
<td>Emotional reactivity</td>
<td>E2</td>
<td></td>
<td></td>
<td></td>
<td>.455</td>
<td>.318</td>
<td>.006</td>
</tr>
<tr>
<td>Placebo</td>
<td>LAmy → LITG</td>
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<td>2.462</td>
<td>.026</td>
<td>.362</td>
<td>.207</td>
<td>.167</td>
</tr>
<tr>
<td>Change in E2 (Day 2 - Day 1)</td>
<td>RAME → RPrec</td>
<td>.254</td>
<td>2.206</td>
<td>.042</td>
<td>.254</td>
<td>.179</td>
<td>.167</td>
</tr>
<tr>
<td>Placebo</td>
<td>Lamy → RITG</td>
<td>.945</td>
<td>.892</td>
<td>.849</td>
<td>.945</td>
<td>.207</td>
<td>.179</td>
</tr>
</tbody>
</table>

Notes. RSMG = right supramarginal gyrus, LIFG = left inferior frontal gyrus, RSMG = right middle frontal gyrus, RSMG = right middle frontal gyrus, RSMG = left inferior frontal gyrus, LSTG = left superior temporal gyrus, LMeFG = left medial frontal gyrus, LSTG = left middle temporal gyrus, LITG = left inferior temporal gyrus, LITG = left inferior temporal gyrus, LAmy = left amygdala, RIFG = right inferior frontal gyrus, RITG = right inferior temporal gyrus, LAMY = left amygdala, LAMY = left amygdala, LPrec = left precentral gyrus, RPrec = right precentral gyrus, RITG = right inferior temporal gyrus.

### 4.4. Stepwise regression results

Stepwise regressions revealed few effective connections that predicted regulation ability and emotional reactivity in the decrease task. The results are summarized in Table 3. Regulation ability after E2 administration was predicted by an inhibitory connection from the RSMG to the LIFG. In the reactivity network within the E2 condition, emotional reactivity was predicted by connections between the amygdala, occipital and temporal regions (RAmy to RPrec, LAmy to LITG, RITG to LAmy) and the change in E2 levels before and after administration. This pattern differed from the results for the placebo condition, where not only connections to and from the left amygdala but also temporal to parietal connections predicted emotional reactivity.
in the prefrontal regions (Khan et al., 2013; Tang et al., 2004). Therefore, one can assume that the PFC is specifically prone to the modulation by E2, increasing cognitive functions and influencing intrinsic network connectivity underlying these functions. However, this observation is not directly supported by the behavioral emotional state ratings that did not differ between the two drug conditions with regard to emotional reactivity and regulation ability. This lack of a group difference in emotion regulation ability might be explained by the relatively small number of total trials (24 trials) and the repetition of stimulus material in the fMRI sessions, which might induce habituation effects. Another aspect contributing to the lack of a behavioral difference may be the choice of short-term exposure to high E2 levels used in this study. We elevated E2 levels from early follicular to pre-ovulatory levels within 24 hrs, similar to Bayer et al. (2018), who also did not observe behavioral changes in a memory recognition task following E2 administration. That a quick rise in one hormone may show physiological effects but lacks an immediate effect on behavior has also been reported for short-term exposure to higher progesterone levels: while an increase in neural activity in the amygdala was detectable, no observable behavioral effect on emotion recognition was reported (Van Wingen et al., 2008).

Next to the increased prefrontal interconnectivity, the temporal and parietal regions demonstrated less effective connectivity to the prefrontal regions in the E2 compared to the placebo condition in the regulation network. Parietal and temporal regions are important integration hubs during the emotion regulation process that have been linked to working memory, attention, and the processing of semantic information (Morawetz et al., 2020). Together with prefrontal regions and the left caudate, the left middle temporal gyrus and left superior temporal gyrus are both part of a meta-analytically derived largescale network on emotion regulation. After E2 administration, we observed stronger inhibitory connectivity from the left medial frontal gyrus to the left middle temporal gyrus and from the left superior temporal gyrus to the left inferior frontal gyrus. Thus, hormonal administration seems to particularly affect intrinsic dynamics within a sub-network primarily linked to cognitive processes, especially language and syntax processing (Morawetz et al., 2020). Resting-state functional connectivity between the superior frontal gyrus and middle temporal gyrus was recently shown to be positively associated with the ability to suppress unwanted intrusive thoughts (Lu et al., 2022). The maintenance of unwanted intrusive thoughts is linked to rumination (Kollarki et al., 2020), the passive repetitive thinking about symptoms of distress and its causes, meanings and consequences (Nolen-Hoeksema et al., 2008). There are several assumptions about how rumination fuels the persistence of unwanted intrusive thoughts, e.g., by changing the appraisals of the intrusive thoughts (Raines et al., 2017), by elevating negative mood, which in turn increases the frequency of intrusive thoughts (Clark, 2002), or by making intrusive thoughts more accessible via the spread of semantic network activation (Grisham and Williams, 2009). Hence, if E2 administration potentially increases the ability to suppress unwanted intrusive thoughts, this might also influence rumination. Rumination has been reported to be more frequently used in women than men (Kuehner, 2017), and recently, a positive association between premenstrual syndrome and rumination has been shown (Nayman et al., 2023). However, the role of E2 for rumination and suppression of unwanted intrusive thoughts in healthy females as well as females suffering from premenstrual syndrome is less clear and could be further investigated in future research.

In terms of the stronger inhibitory connectivity from the left superior temporal gyrus to the left inferior frontal gyrus, previous research has shown that activation and connectivity of these two regions are of paramount importance for auditory cortical processing and integration of language and action, in particular speech and non-speech stimuli (Willems et al., 2009), thereby heavily relying on the arcuate fasciculus as shown by data from female and male patients with medically intractable epilepsy (Garell et al., 2013). Regarding prefrontal-parietal connectivity, we observed a decreased inhibition of the right supramarginal gyrus on the left inferior frontal gyrus following E2 administration.

Moreover, the stepwise regression analysis revealed that the ability to decrease negative emotions after E2 administration was predicted by the inhibitory connection from the right supramarginal gyrus to the left inferior frontal gyrus. The right supramarginal gyrus is mainly involved in shifting attention to salient stimuli (Shulman et al., 2007). It is part of the ECN that is specialized in higher cognitive control functions (Seeley et al., 2007). The insight that effective connectivity within the ECN and, in particular, between right supramarginal gyrus and prefrontal regions is influenced by the menstrual cycle phase has been gained recently (Hidalgo-Lopez et al., 2021). There, increased effective connectivity between right supramarginal gyrus and left prefrontal regions occurred when comparing the pre-ovulatory to the early follicular phase, thus when their female participants were in the high E2 phase. A similar effect was also reported for the comparison of the luteal to the early follicular phase, indicating that effective connectivity between right supramarginal gyrus and left PFC regions may be particularly sensitive to high hormonal states, potentially influencing higher cognitive control via attention shifts in an inhibitory vs. excitatory fashion thereby influencing behavioral performance.

Taken together, our data suggest that high levels of E2 change intrinsic dynamics within prefrontal regions but also (inhibitory) connectivity with specific temporal and parietal regions, thereby potentially enabling an intrinsic state that may prepare females for the upcoming (emotion regulation) task (Rebbein et al., 2021). Our findings point towards stable regulation ability that comes at lower costs by decreasing the neuronal effort in terms of changes in connectivity and activity. Hence, these results provide the first evidence for a preparatory state of the reappraisal process that enables females during phases with higher E2 levels to more efficiently regulate their emotions since effective connectivity within prefrontal regions has been associated with reappraisal ability (Morawetz et al., 2017).

Regarding the reactivity network, we also observed effects of E2 administration, which are manifold and complex. Especially connectivity to and from the left inferior temporal gyrus and the right inferior parietal lobe to the left precuneus was altered by E2 administration, thus indicating that E2 influences the input and output of regions that integrate information from multiple other brain regions. Functional activation and connectivity of the inferior temporal gyrus and the inferior parietal lobe have previously been reported to be influenced by the menstrual cycle (Dubol et al., 2021). Positive correlations of E2 levels with intrinsic functional connectivity of the inferior parietal lobe (Hidalgo-Lopez et al., 2020), the functional connectivity between the inferior parietal lobe and ECN (De Bondt et al., 2015) as well as functional connectivity between the inferior parietal lobe and the inferior frontal gyrus during sadness induction (Dan et al., 2019) have been reported. Moreover, during response inhibition, functional connectivity between the left inferior parietal lobe and the right middle frontal gyrus was stronger in the early follicular phase than in the mid-luteal phase (Thimm et al., 2014). Weis et al. (2011) showed reduced effective connectivity of the right intertemporal gyrus with precuneus, prefrontal regions and cerebellum during the peri-ovulatory and the mid-luteal phase, further supporting the notion that activation and connectivity of the intertemporal gyrus are influenced by E2 levels. The way we react to emotional images might thus be influenced by E2 levels through changes in the intrinsic dynamics of these regions. The temporal and parietal regions demonstrated modulated effective connectivity between them. In addition, these regions were less connected with the left precuneus, which projects to the right precuneus. The right precuneus, in turn, shows an increased inhibitory reciprocal connection to the amygdala, which shows enhanced excitatory connectivity to the temporal cortex. While the parietal, temporal and frontal regions have previously been placed into different large-scale neural networks underlying emotion regulation, see also discussion above, the amygdala is
a crucial region involved in emotional reactivity (Morawetz et al., 2020).

The behaviorally reported emotional reactivity after E2 administration was further predicted by connections from and to the amygdala. These findings suggest a possible neural pathway between integration hubs and key emotion processing regions like the amygdala linked to the generation and experience of emotions. In terms of amygdala connectivity, previous studies reported stronger functional connectivity of the amygdala with cingulate cortex and right angular gyrus (left amygdala seed) as well as left middle temporal gyrus (right amygdala seed) during the mid-follicular than late luteal phase (Petersen et al., 2019). Comparing the early follicular to mid-luteal phase, Engman et al. (2018) reported stronger connectivity of the left and right amygdala to prefrontal regions as well as the cerebellum and paracentic lobule (left amygdala seed). However, a very recent cross-sectional study, including E2 administration, did not report a significant influence on functional connectivity of the amygdala in their mixed sample of females and males (Coenjaerts et al., 2023). In our study, we observed several changes in the effective bilateral connectivity of the amygdala, especially with the right precentral in terms of inhibitory connections, only during E2. Also, excitatory connectivity from right to left amygdala was stronger under E2. Increased connectivity between the amygdala and the precentral has been reported when female and male participants were instructed to focus on non-arousing regions of unpleasant images compared to natural viewing (Ferri et al., 2016). In this study, amygdala-precentral connectivity correlated positively with eye-tracking measures of attentional deployment success and with trait reappraisal, suggesting that the connectivity between these two regions might relate to the ability to successfully implement attentional deployment and the predisposition to utilize adaptive emotion regulation strategies.

4.1. Limitations and future research

We note that there are several limitations to consider when interpreting our findings. As previously noted in Rehbein et al. (2021), having the examination date take place in the female’s early follicular phase was an attempt at isolating E2 effects from the impact of other sex steroids. Yet, ovarian hormones are always intertwined and can hardly be studied entirely independently from one another. Future studies may consider studying endogenous ovarian hormone fluctuation across the menstrual cycle (Hidalgo-Lopez et al., 2021) also including females reporting irregular menstrual cycles or premenstrual syndrome, females using hormonal contraception (Hidalgo-Lopez et al., 2023) or undergoing hormonal transition periods, e.g., menopausal transition, or clinical groups including females diagnosed with endometriosis, polycystic ovary syndrome, etc. to foster our understanding of the interaction of neuroendocrine action on brain dynamics.

Another limitation is the questionable transferability to everyday situations. This study showed explorative effects under lab conditions that still need further research to show an extension into daily life and natural emotion processing. Especially when discussing the role that E2 administration could play in treatments, further research is essential to assess the (side) effects this has outside of lab conditions. Besides the endogenous variation within the menstrual cycle and endogenous or synthetic E2 included in some oral contraceptives, administration of E2 is prescribed in gender affirming hormone therapy and menopausal hormone replacement therapy, thus millions of women worldwide are faced with some kind of E2 administration. Here we only investigated a short-time boost of E2 levels, however, most of the above-mentioned therapies rely on long-term intake and data on how these affect emotion regulation and its neural networks is scarce. E2 has neurotrophic and neuroprotective effects that also affect neuropsychological processes such as memory formation, particularly via promotion of cell growth and cell survival of hippocampal and basal forebrain neurons (Morgan et al., 2018). In aging, memory function is affected by shifts in estrogen receptor ratio subsequently influencing estrogen-regulated gene transcription (Foster, 2012). And, following the critical window hypothesis (Maki, 2013), exogenous E2 may support glycolytic metabolism and via its influence on insulin-regulating mechanisms may promote cell health particularly in areas vulnerable to Alzheimer disease pathology (Schioth et al., 2012).

4.2. Conclusion

In conclusion, our study is the first to reveal how resting-state dynamics within emotion regulation-related networks relate to behavioral measures of emotional reactivity and reappraisal ability in association with estradiol (E2) concentration. In general, we found that higher levels of E2 are related to changes in effective resting-state connectivity within both networks. More specifically, the high degree of interconnectivity within prefrontal regions on the one side and the changes in baseline effective connectivity from temporal and parietal regions to the prefrontal cortex on the other side might support the ability to effectively and efficiently regulate negative emotions during phases of high levels of E2. Our study represents a first step in understanding the causal interplay of emotion regulation-related brain regions at rest and how this might relate to behavioral effects – which in itself is an insightful extension of brain activity studies like Rehbein et al. (2021). Moreover, investigating the impact of ovarian hormones on human brain coupling via effective connectivity offers a window into understanding the underlying neuroendocrine interactions across various hormonal transition periods and developmental trajectories. This would lay the basis for future studies interested in the mechanisms underlying the increased vulnerability for mental health issues of females during phases of hormonal transition. As an example, consideration of endogenous E2 (and its potential modification via hormonal administration) could play a supporting role in the treatment of mental disorders that show a dysregulation of emotions.

CRediT authorship contribution statement

Birgit Derntl: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Corinna H Eber: Writing – review & editing, Formal analysis. Lydia Kogler: Writing – review & editing, Supervision, Project administration, Methodology, Investigation. Elisa Rehbein: Writing – review & editing, Methodology, Investigation, Data curation. Inger Sundstrom-Poromaa: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Carmen Morawetz: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization.

Declaration of Competing Interest

None.

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References

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