Hippocampal volume and affect in response to fluctuating estrogens in menstrual cycle irregularity: A longitudinal single-subject study

Carina Heller (carina.heller@uni-jena.de)
Friedrich Schiller University Jena

Daniel Güllmar
Jena University Hospital

Carina J. Koeppel
Friedrich Schiller University Jena

Philine Rojczyk
Harvard Medical School

Heidemarie Stein
Friedrich Schiller University Jena

Caitlin M. Taylor
University of California, Santa Barbara

Emily G. Jacobs
University of California, Santa Barbara

Birgit Demtl
University of Tübingen

Zora Kikinis
Harvard Medical School

Martin Walter
Jena University Hospital

Ilona Croy
Friedrich Schiller University Jena

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Abstract

Background

The menstrual cycle is a critical indicator of women's reproductive, physical, and mental health, influenced by neuromodulatory sex steroid hormones, including estrogens like 17β-estradiol, and estrone. Irregular menstrual cycles can lead to various health conditions. Understanding the relationship between endogenous hormone fluctuations and brain function across the menstrual cycle is essential for comprehending mental health disorders prevalent in women. Here, we investigated the impact of hormonal variations on hippocampal morphology and affect in a participant with an irregular menstrual cycle.

Methods

In this dense-sampling longitudinal study, a healthy female with an irregular menstrual cycle underwent testing for five consecutive weeks, covering mostly the follicular phase and ovulation. Daily blood draws provided measurements of estradiol, estrone, and progesterone. T₁-weighted MRI scans assessed bilateral hippocampal volumes. Psychological measures of positive and negative affect were collected each session. Statistical analyses included cubic regression curves, Spearman correlations, and mediation regression models to explore hormonal associations with hippocampal morphology and affect.

Results

Fluctuations were observed in hormonal concentrations, hippocampal volume, and affect across the 25 testing days. Estradiol and estrone correlated significantly with hippocampal volume, while progesterone did not show any significant association. Increased estrogen levels were linked to decreased positive affect, mediated by hippocampal volume fluctuations. Increased estrogen levels were further associated with increased negative affect, however, independently of hippocampal changes.

Conclusion

This study sheds light on the complex relationship between endogenous hormone fluctuations, hippocampal morphology, and affect in a participant with an irregular menstrual cycle. The findings suggest potential roles of estrogens and estrone in affect regulation, with implications for women's mental health and brain function. Further research is warranted to explore these associations in larger samples and various menstrual cycle patterns.

1. Introduction
The menstrual cycle is an important indicator for reproductive, physical, as well as mental health of women. Estrogens, including estradiol, estrone, and estriol, constitute a major class of neuromodulatory sex steroid hormones. Estradiol is the most potent estrogen in humans [1] and is approximately 10 times as potent as estrone and 80 times as potent as estriol [2]. For most women, a regular menstrual cycle ranges from 21 to 35 days and women exhibit sinusoidal variation in serum estradiol, consisting of the follicular phase (~ 10–22 days in duration) and the luteal phase (lasting ~ 14 days) [3]. The follicular phase is mainly dominated by estradiol, with the low levels during the early follicular phase and peak concentrations in the peri-ovulatory period. A smaller peak in estradiol appears during the mid-luteal phase [4, 5]. Both estrone and estriol are precursors and metabolites of estradiol [6, 7]. In contrast to estradiol, progesterone follows a bimodal response with very low concentrations during the follicular phase, and high sustained values for most of the luteal phase of the menstrual cycle [8]. However, 5 to 36% of women are affected by irregular menstrual cycles, depending on age, country of residence, or occupation [9]. There are multiple etiologies for menstrual cycle irregularities, such as a dysfunction of the hypothalamic-pituitary-ovarian axis which influences the production of estradiol. Research suggests that prolonged and irregular cycle length are associated with decreased estradiol exposure [10] and, in this line, with an increased risk for cardiovascular diseases, osteoporosis [11], type 2 diabetes mellitus [12], and a decreased risk for ovarian cancer [13]. It should be noted that 69% of the variance in total menstrual cycle length is due to the variance of the follicular phase length when estradiol is the dominating hormone. Only 3% of the variance in the total length of the menstrual cycle is due to the length of the luteal phase when progesterone is dominant [3, 14]. Hence, estradiol is considered the most important hormone in regulating the menstrual cycle, particularly the length of the follicular phase.

The brain is an important target for estrogen and progesterone [15, 16]. While cells in many brain regions express estrogen and progesterone receptors, an increased presence of receptors is found in the hippocampus ex vivo in humans [17] and in rodents [18]. It is well known that the hippocampus plays a major role in memory and control of attention. Investigating the effect of endogenous estrogen and progesterone on hippocampal neuroplasticity in humans in vivo is a relatively recent scientific effort. Currently, insights into brain-hormone investigations in humans are based predominantly on cross-sectional designs. However, as hormonal concentrations change across the menstrual cycle, longitudinal studies that track individuals over time are of particular importance. Following this, a series of recent neuroimaging studies have tracked women across the menstrual cycle over periods of weeks and months [19–26] to enrich our understanding of hormone action in the human brain. Barth et al. (2016) conducted a longitudinal study of a single woman to examine fluctuating hormone levels during the natural cycle and corresponding structural changes in the hippocampus [19]. Thirty MRI scans were acquired every two to three days in two separate scanning session over the course of two complete menstrual cycles. They observed a positive association between endogenous estrogen concentrations and bilateral white matter hippocampal fractional anisotropy, an indicator of the microstructural properties in white matter [27]. The interplay between regional brain volume and hormonal levels was also demonstrated in a longitudinal study of one woman in which fluctuating hormone levels and hippocampal subfield volumes were examined every 24h across 30 consecutive days across a complete menstrual cycle. Endogenous
progesterone concentrations were related to gray matter volume in the hippocampal subfields of CA2/3 and paralimbic structures of the parahippocampal gyrus, perirhinal and entorhinal cortex. Subsequent pharmacological suppression of progesterone eliminated these effects [20]. No significant associations with estradiol were reported. These findings were reported in women with a regular menstrual cycle but it is unknown whether the relationship is present in individuals with an irregular cycle.

The hippocampus is implicated in mental health disorders such as depression [28]. Women are approximately twice as likely to be diagnosed with major depressive disorder (MDD) compared to men [29], and fluctuations of ovarian hormones are related to depression susceptibility and prevalence in women [30, 31]. Most interestingly, menstrual cycle irregularities, in addition to physiological factors, have been associated with mental health conditions, including depression [32]. Across the menstrual cycle, women reported increased non-pathological psychological symptoms related to neuroticism and depression from the late follicular phase (when estradiol rises and progesterone is still low) to the late luteal phase (when both hormone concentrations are declining) [33]. However, a longitudinal analysis of hormonal levels and mood across the menstrual cycle in a healthy sample found only negligibly effects of fluctuating hormone levels on affective states [34]. Due to diverging results on sex hormones in association with affect, it is yet unknown whether low or high levels of endogenous hormones cause changes in mood and affect. Inconsistent findings could be explained by inconsistent operationalization methods of the menstrual cycle [14]. Since menstrual cycle length varies between individuals (the average is between 21 and 37 days) and within individuals cycle-to-cycle [35–37], self-report assessments of menstrual cycle stage are often misleading. Further, saliva-based assessments are subpar [38]. Following this, the menstrual cycle is fundamentally a within-person process. Inconsistencies reported in previous studies could be overcome by applying repeated measure designs, which should be seen as the state-of-the-art approach [14]. However, within-subject designs are challenging in terms of the demands placed on the participant. Therefore, these dense-sampling studies, as described above, are still scarce.

Here, in a dense-sampling study of one female participant with an irregular menstrual cycle, we explored whether endogenous fluctuations in sex hormone concentrations impact hippocampal morphology and affect. First, we investigated associations of endogenous concentrations of estradiol, estrone, and progesterone with hippocampal morphology across five consecutive weeks ($n = 25$ testing days) covering mostly the follicular phase and ovulation. Second, we determined the association between the concentrations of circulating hormones, hippocampal morphology, and affect.

2. Methods

2.1. Participant

A healthy female (30 years of age) participated in this dense-sampling, longitudinal study. The participant underwent testing from Monday to Friday for five consecutive weeks (August 2nd – September 2nd, 2022) while freely cycling, resulting in $n = 25$ test sessions. The female participant was free from
hormonal medication for 55 months (mean menstrual cycle length = 38.3 days, SD = 6.68 days during that time) before the assessment. The female participant had no history of psychiatric, neurological, and endocrine diagnoses, breastfeeding or pregnancy, and no history of smoking, alcohol, or drug abuse. The participant gave written informed consent, and the Friedrich Schiller University Jena Ethics Committee approved the study.

2.2. Image acquisition and postprocessing

The imaging data were acquired on a 3T Siemens PrismaFit scanner (Siemens Medical Solutions, Erlangen, Germany) with a 64-channel head coil. Structural MRI were acquired with $T_1$-weighted (T1w) MPRAGE sequence with GRAPPA acceleration. Scan parameters were: echo time (TE) = 2.22 ms, repetition time (TR) = 2400 ms, (TI) = 1000 ms, matrix size = 256 x 256, field of view (FOV) = 256 mm, band with = 220 Hz/pixel and slice thickness = 0.80 mm. Scans were collected every day at 7.30 am local time. The parameters used to acquire the images (e.g., sizes, space directions, space origin), and the quality of the images (e.g., motion artifacts, ringing, ghosting of the skull or eyeballs, cut-offs, signal drops, and other artifacts) were visually checked. Sequence Adaptive Multimodal SEGmentation (SAMSEG) [39] was used to segment both hemispheres' total hippocampal volumes. Initially, a subject-specific template was created by spatially co-registering all 3D T1w MRPAGE volumes through an iterative process [40]. The co-registered 3D volumes were then employed to implement longitudinal SAMSEG [41]. The final segmentations were used to extract the volume of the hippocampus structure directly for each measurement day. The numerical values of the left and right hippocampal volumes were demeaned by dividing each individual value of both hemispheres by the hemisphere mean, for both hemispheres respectively. The demeaned hippocampal volumes were then added and divided by two to obtain a ratio.

2.3. Endocrine procedure

The blood was drawn at 9.00 am. One 7.5 ml blood sample was collected in a S-Monovette® Serum-GEL (Sarstedt) with clotting activator/gel each test session. The sample clotted at room temperature and was stored at 5° Celsius until centrifugated (2500 x g for 10 minutes). Estradiol (pg/ml) and progesterone serum concentrations (ng/ml) were determined at the Bioscientia Laboratory in Jena, Germany. Estrone serum concentrations (pg/ml) were determined at the Bioscientia Laboratory in Ingelheim, Germany. Estradiol was assessed with the electrochemiluminescence immunoassay (ECLIA) Elecsys® Estradiol III Assay. Assay antibodies, measuring ranges (defined by the limit of detection and the maximum of the master curve), and intra-assay coefficients of variation for estradiol were the following: antibodies, two biotinylated monoclonal anti-estradiol antibodies (rabbit), 2.5 ng/ml and 4.5 ng/ml; measuring range, 18.4–11,010 pmol/l (5–3000 pg/ml), < 5% relative SD. Progesterone was assessed with the ECLIA Elecsys® Progesterone III Assay. Assay antibodies, measuring ranges, and intra-assay coefficients of variation for progesterone were the following: antibodies, biotinylated monoclonal anti-progesterone antibody (recombinant sheep), 30 ng/ml; measuring range, 0.159–191 nmol/l (0.05–60 ng/ml), < 5% relative SD. All assays were determined on the cobas® e 801 analyzer (Roche Diagnostics GmbH,
Mannheim, Germany) and were used according to the manufacturer's instructions. Radioimmunoassay (RIA) was used to determine concentrations of estrone.

2.4. Psychological measures

Positive and negative affect was assessed for each test session separately using the Positive and Negative Affect Schedule (PANAS) [42]. The PANAS is a 20 item self-reporting questionnaire assessing positive emotions such as joy, interest, and alertness, and negative emotions such as sadness, distress, and irritability. Each item on the PANAS is rated on a 5-point scale, ranging between 1 and 5, with 1 indicating low agreement to the specific item (not at all) and 5 indicating a high agreement (very much). The positive affect score was calculated as the average of the 10 positive items. The negative affect score was calculated as the average of the 10 negative items. Hence, positive and negative affect scores can range from 1 to 5. Lower scores represent lower levels of positive and negative affect, whereas higher scores represent higher levels of positive and negative affect, respectively.

2.5. Statistical approach

Statistical analyses were performed using R software (https://www.r-project.org), Statistical Package for Social Sciences (SPSS) version 27, and GraphPad Prism 8. First, cubic regression curve estimations were used as the data followed a cubic curve to check whether hormones, hippocampal volume, and affect fluctuated significantly across the 25 testing sessions.

Second, Shapiro-Wilk’s test was used to check for the normal distribution of the variables. As hormonal concentrations were not normally distributed, Spearman correlations were performed between hippocampal volumes, hormones, and positive and negative affect. False Discovery Rate (FDR) correction was used to correct for multiple comparisons in all analyses [43].

Third, in case of a significant correlation, we used mediation regression analyses to investigate whether fluctuations in positive and negative affect were a direct effect of fluctuations in hormonal concentrations across the 25 test sessions or an indirect effect mediated by fluctuations in left and right hippocampal volume. Mediation regression models were calculated with positive and negative affect as dependent variables and hormonal levels as independent variables. Hippocampal volumes were added as a mediator variable to the model. Due to high multicollinearity among the independent variables the analyses were conducted separately for each hormone, hippocampal hemisphere, and positive and negative affect. In our mediation regression models, path $a$ is the linear effect of the hormonal levels (independent variable) on hippocampal volume. Path $b$ is the effect of hippocampal volume (mediator) on positive and negative affect (outcome variables). The indirect effect $ab$ measures the amount of mediation, and the direct effect $c$ is the effect of the hormonal levels on positive and negative affect after controlling for hippocampal volume. The total effect $c$ is the sum of direct and indirect effects. Results were based on 5000 bootstrapped samples. Residuals of the regressions were normally distributed.

3. Results
3.1. Analysis I: Fluctuations across the 25 test sessions

Hormonal concentrations (estradiol: $F(3,21) = 6.698, p = 0.002$; estrone: $F(3,21) = 14.728, p < 0.001$; progesterone: $F(3,21) = 46.306, p < 0.001$), hippocampal volume ($F(3,21) = 5.574, p = 0.006$), and affect (positive affect: $F(3,21) = 17.604, p < 0.001$; negative affect: $F(3,21) = 13.986, p < 0.001$) fluctuated significantly across the 25 testing days covering mainly the follicular phase and ovulation (see Fig. 1). The menstrual cycle at the time of the scan lasted 53 days, which represented a longer irregular menstrual cycle than usual ($M = 38.3$ days, $SD = 6.36$ days during the 55 hormone-medication-free months prior to the study). Ovulation was confirmed through ovulation tests and luteinizing hormone (LH) blood concentrations. According to the results, ovulation occurred on testing days 21 and 22, representing menstrual cycle days 37 and 38. Following this, the study covered 20 days of the follicular phase, 2 days of ovulation, and 3 days of the luteal phase. The luteal phase of this menstrual cycle covered 15 to 16 days in total.

3.2. Analysis II: Hormonal concentrations in association with hippocampal volume and affect

Next, we tested whether hormone-brain-behavior fluctuations were associated with each other. Bilateral hippocampal volume correlated significantly with estradiol ($r = 0.637, p = 0.001$, $FDR = 0.002$) and estrone ($r = 0.745, p < 0.001$, $FDR < 0.001$) but not with progesterone ($r = -0.036, p = 0.863$, $FDR = n.s.$).

Both estradiol and estrone correlated significantly with positive (estradiol: $r = -0.469, p = 0.018$, $FDR = 0.026$; estrone: $r = -0.427, p = 0.033$, $FDR = 0.040$) as well as negative affect (estradiol: $r = 0.773, p < 0.001$, $FDR < 0.001$; estrone: $r = 0.661, p < 0.001$, $FDR < 0.001$), suggesting that increased estrogen concentrations were associated with decreased positive but with increased negative affect. Progesterone correlated significantly with positive ($r = 0.464, p = 0.019$, $FDR = 0.026$) but not with negative affect ($r = -0.021, p = 0.919$, $FDR = n.s.$), suggesting that increased progesterone concentrations were associated with decreased positive affect.

Furthermore, positive affect was significantly inversely associated with bilateral hippocampal volume ($r = -0.681, p < 0.001$, $FDR < 0.001$), whereas negative affect was significantly associated with bilateral hippocampal volume ($r = 0.485, p = 0.014$, $FDR = 0.026$).

3.3. Analysis III: Estrogens are linked to negative affect, hippocampal volume is linked to positive affect

We used mediation regression analyses to investigate whether positive and negative affect were a direct effect of hormonal concentrations across the 25 test sessions or an indirect effect mediated by fluctuations in hippocampal volume.

For the outcome variable positive affect, model 1.a included estradiol as the independent variable and hippocampal volume as mediator. For model 1.b, estradiol was replaced by estrone as independent
variable. Neither estradiol nor estrone were identified as significant predictors since total effects $c$ and direct effects $c'$ were insignificant. The indirect effect $a*b$ for hippocampal volume was significant in both models, suggesting that bilateral hippocampal volumes were related to positive affect rather than estrogens. Figure 2 shows the detailed results for mediation analysis model 1.a and 1.b.

For negative affect, models 2.a included estradiol as the independent variable and hippocampal volume as mediators. Since total effects $c$ and direct effects $c'$ were significant, estradiol was identified as a significant predictor for negative affect. Model 2.b included estrone as the independent variable, negative affect as the outcome variable, and hippocampal volume as mediator. Similar to estradiol, the total effects $c$ and direct effects $c'$ of estrone predicted negative affect. The indirect effect $a*b$ for hippocampal volume was not significant in both models, suggesting that negative affect was directly related to estrogen concentrations and was not mediated by bilateral hippocampal volumes. Figure 3 shows the detailed results for mediation analysis 2.a and 2.b.

4. Discussion

The hormonal concentrations of estrogens and progesterone vary across the female menstrual cycle. Estradiol is the dominant hormone during the follicular phase of the menstrual cycle, while progesterone dominates the luteal phase [44]. In this dense-sampling study of one female participant with an irregular menstrual cycle, we link these endogenous endocrine changes to changes in bilateral hippocampal volume. Here, estrogen concentrations were positively associated with bilateral hippocampal volume during a prolonged follicular phase. The results are consistent with Barth et al. (2016) reporting increased volumetric fractional anisotropy (FA) in the bilateral hippocampus associated with increased estradiol across the full menstrual cycle [19]. Moreover, our results are consistent with cross-sectional data reporting lower hippocampal volumes in the early follicular phase and increased hippocampal volume during the late follicular phase of the menstrual cycle [45, 46]. As was the case for our study, Barth et al. (2016) did not report hippocampal volume associations with progesterone [19]. This is inconsistent with the reported results of associations between hippocampal volume and progesterone by Taylor et al. (2020) [20]. One explanation for the missing associations between progesterone and hippocampal volume in our study could be that the 25 test sessions in the current study only covered three days of the luteal phase of the menstrual cycle when progesterone concentrations are dominant. The majority of test sessions covered the follicular phase as well as ovulation when progesterone is, overall, low. Another explanation would be that Taylor et al. (2020) performed correlations between progesterone concentrations and volumes of hippocampal subfields, such as CA1, CA2/3, dentate gyrus, whereas Barth et al. (2016) performed whole-volume hippocampal correlations [19, 20]. The dentate gyrus of the hippocampal formation is one of the few brain areas that may exhibit adult neurogenesis. Therefore, the background of the observed structural changes in the hippocampal formation might be linked to alterations in dendritic branching or neuronal cell growth [47]. The underlying mechanisms by which sex hormones and hippocampal morphology are linked still need to be elaborated. Published results to date indicate that hormonal fluctuations across the menstrual cycle as well as during an irregular prolonged follicular phase impact hippocampal morphology. While regular menstrual cycles of ~ 28 days are
associated with an increased estradiol exposure, longer irregular menstrual cycles are associated with an
decreased estradiol exposure as estradiol concentrations remain lower for a longer period of time [10]. In
the context of the current study, this implies that decreased hippocampal volumes are associated with
prolonged irregular cycles.

In addition to variations in hormonal patterns and bilateral hippocampal volumes, we report significant
positive and negative affect fluctuations across the 25 test sessions. Positive and negative affect were
significantly associated with both estrogen and hippocampal volume. Decreased positive emotions were
significantly associated with increased estrogen concentrations, suggesting that positive emotions such
as joy and interest were low during phases when estrogens are high, such as during the late follicular
phase and ovulation. However, the indirect effect of the hippocampal volume mediated this relationship.
This suggests that fluctuations in positive affect are not directly related to fluctuating estrogens but
rather to fluctuating hippocampal morphology. Increased negative emotions, such as sadness and
irritability, were associated with increased estrogen concentrations. This suggests that during phases
when estrogens are high, such as late follicular phase and ovulation, negative affect was high. Unlike
positive affect, the association between increased negative affect and estrogens was not mediated by an
indirect effect of hippocampal volume. It should be noted that in our study the negative affect scores
across the 25 test sessions were non-pathological and low in general since the study was conducted in a
healthy participant. However, the findings suggest that fluctuations in negative emotions were better
explained by estrogens than by fluctuations in hippocampal morphology. Our current results of increased
negative affect and decreased positive affect when estrogen levels are higher are in stark contrast to
previous studies and the widespread assumption of rather reporting more negative affect premenstrual
and more positive affect around ovulation [33, 48–50]. In this line, Taylor et al. (2020) reported
progesterone fluctuations over the full menstrual cycle to be associated with measures of anxiety,
tension, depression, and confusion [20]. However, there is also evidence that ovarian hormones make little
or no contribution to daily mood and affective variability in naturally cycling women [34, 51–54]. Given
that these studies had controlled for irregular menstrual cycles, our results may suggest an opposing
mechanism of affect and hormonal fluctuations in menstrual cycle irregularities. However, it should be
noted that studies reporting increased positive mood during times of ovulation and increased negative
mood in the luteal phase often do not report hormonal concentrations measured in blood or saliva [33,
48, 49].

The results of the current study may provide insight into the endocrine factors that underlie increased
susceptibility and prevalence of depression in women [30, 31]. Women are twice as likely to be diagnosed
with depression compared to men [29] and this increased susceptibility is seen only during the
reproductive years while the prevalence of depression in prepuberty and after the age of 55 is almost the
same in men [55]. The menstrual cycle is an important indicator for reproductive, general, as well as
mental health of women [56]. Menstrual cycle irregularities have been associated with mental health
conditions [32] and irregular menstrual cycle variability before pregnancy was reported to be associated
with depression during pregnancy [57]. The findings of our study in an irregular menstrual cycle highlight
the role of estradiol and estrone in negative affect, which is thought to be a vulnerability factor for
depression [58, 59]. Moreover, the results suggest that short-term changes in hippocampal volume within 25 test sessions influence the perception of less positive emotions. The decline in positive affect was associated with depression in the past [58], as have volume changes in the hippocampal formation [47]. We cannot provide conclusive interpretations for these associations given that our findings are based on one healthy participant with menstrual cycle irregularities. However, the question arises as to what function these short-term changes serve across the menstrual cycle and whether fluctuations in estrogens and hippocampal volume act as a protective factor or risk factor for developing depressive disorders in some women. The results of this study suggest that we need to continue to investigate the influence of the regular menstrual cycle but also the influence of the irregular menstrual cycle on the brain and mental health.

Little is known about estrone's role in perimenopause as it was more studied in postmenopausal women and, to date, there are no studies on estrone, negative affect and brain health in premenopausal women. It should be noted that estrone is a precursor to estradiol. Our results indicate its potential role, besides estradiol, on affect during the (irregular) menstrual cycle. It remains unclear whether the role of estrone in hippocampal morphology and affect is uniquely in women with an irregular cycle or whether it has the same impact in women with a regular menstrual cycle of ~ 28 days.

4.1. Limitations and future directions

Several limitations to our study must be noted. First, although no endocrine condition or diagnosis was known before scanning, the participant had a 53-day menstrual cycle during the 25 testing sessions. A local gynecologist ruled out a diagnosis of Polycystic Ovarian Syndrome (PCOS). Further hormonal analyses, ordered by the gynecologist, revealed increased prolactin levels of unknown origin after scanning. Hyperprolactinemia is the most common pituitary hormone hypersecretion syndrome, which most commonly affects women between the ages of 25 and 34 [60], with women often reporting unregular menstrual cycles [61]. Given that stress influences prolactin secretion in humans [62], it is possible that prolactin serum concentrations were elevated due to the stressful procedures of daily MRI scans and blood draw. Following this, the results could also be explained by increased or fluctuating prolactin concentrations, which was not assessed during this study.

Second, due to a longer menstrual cycle of overall 53 days during the scanning sessions, we were not able to scan the participant across a complete (irregular) menstrual cycle which usually consists of menses, follicular phase, ovulation, and luteal phase. Future studies should continue dense-sampling studies with complete menstrual cycles. However, when conducting in vivo research with humans, unexpected events like a prolonged menstrual cycle are not always predictable or avoidable. Despite striking differences in cycle length between densely-sampling participants, the consistency of our findings with previous reports underscores the robustness of these associations.

Third, one of the authors was the participant in this study and it was not possible to conduct a blinded study. As a result, responses to the questions on the positive and negative affect scores may have been
influenced by the knowledge that individuals, for example, might experience premenstrual symptoms such as stress anxiety, fatigue, mood swings, anxiety, or depression shortly before the end of the menstrual cycle [63]. However, this is unlikely given that the participant experienced an irregular menstrual cycle with a total length of 53 days at the time of scanning. Therefore, the participant was not fully aware of which phase she was in, since hormonal levels were not revealed until after completion of the test sessions.

Lastly, since this study is a longitudinal study with a dense single-subject design, interpretations and explanations of the reported relations should be made with caution as no causal effects can be generalized to larger populations. Given that our study is the first to report the interplay of estrogen fluctuations with hippocampal volumes and positive and negative affect across a dense-sampling study of five weeks in an irregular menstrual cycle, further studies are needed to replicate and extend these results. Furthermore, scores on positive and negative affect were non-pathological since the study was conducted with a healthy participant without a history of mental health diagnoses. Future studies could include female participants diagnosed with MDD to clarify whether the influence of the menstrual cycle and its hormonal fluctuations is different in individuals with and without clinical depression.

The strength of this study was its dense daily measurement time resolution over a total of five weeks to investigate macrostructural changes in hippocampal volume in the brain under the influence of hormones of the female menstrual cycle (irregularities). Compared to this study, Barth et al. (2016) [19] acquired MRI scans every second or third day in two separate scanning sessions covering two full menstrual cycles. Because this study revealed changes in the hippocampus across female menstrual cycle irregularities and Taylor et al. (2020) [20] reported changes across a regular menstrual cycle, it would be beneficial to examine male participants over five weeks and determine sex hormones, such as estradiol, progesterone, and testosterone, to clarify whether these changes are unique to women. Furthermore, investigating female participants with a diagnosis of MDD would shed light on whether fluctuating hormones and hippocampal volumes are associated with an increased susceptibility to depressive symptoms in women.

4.2. Conclusion

In conclusion, this dense-sampling study provides valuable insights into the intricate interplay between endogenous hormone fluctuations, hippocampal morphology, and affect in a participant with an irregular menstrual cycle. The findings highlight the significant associations of estradiol and estrone with bilateral hippocampal volume, suggesting potential hormonal contributions to brain structure. Moreover, estrogen levels were linked to affect, revealing their role in influencing positive and negative emotions. The study's focus on an irregular menstrual cycle emphasizes the importance of investigating hormone-brain relationships beyond regular cycles, shedding light on potential implications for mental health disorders prevalent in women. However, these results are based on a single participant, warranting caution in generalizing findings to the broader population. Further research with larger and diverse samples is necessary to validate and expand these findings, elucidating the mechanisms underlying hormonal influences on brain health and affect regulation in women.
Declarations

Author contributions:

CH was responsible for the study concept and design, acquired the MRI data, blood samples, and psychological questionnaires, processed and analyzed the data, performed the statistical analysis, and wrote the manuscript. DG acquired, processed and analyzed the MRI data, and supervised the inspection of the anatomical data. CJK and HS collected the blood samples and were involved in the critical revision of the manuscript. PR, CMT, EGJ, and BD assisted with the interpretation of the findings and were involved in the critical revision of the final manuscript. ZK, MW, and IC assisted with the study concept and design and were involved in the critical revision of the final manuscript.

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Competing interest:

All authors declare no financial or non-financial competing interests.

Data availability:

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

References


Figures

Figure 1

Hormonal concentrations, hippocampal volume, positive and negative affect changes across 25 testing days. Estradiol, estrone, and progesterone concentrations are displayed across the 25-day experiment.
Changes in hippocampal volume, positive and negative affect across the experiment are displayed. Note that ‘Test Day 1’ refers to ‘Cycle Day 10’. Ovulation occurred on testing days 21 and 22 which refer to cycle days 37 and 38. Hormone icon pictogram, source: iStock. Licensed under the standard license.

Figure 2

Mediation analysis of hormonal levels, positive affect, and hippocampal volumes. Path a is the linear effect of the hormonal levels (independent variable) on hippocampal volume. Path b is the effect of
hippocampal volume (mediator) on positive affect (outcome variable). The indirect effect $a \times b$ measures the amount of mediation, and the direct effect $c'$ is the effect of the hormonal levels on positive affect after controlling for hippocampal volume. The total effect $c$ is the sum of direct and indirect effects. All paths’ estimates are depicted as regression coefficients, respective p-values and 95% confidence interval (95%CI). Significant results are indicated in bold. n.s. = non-significant. Hormone icon pictogram, source: iStock. Licensed under the standard license.

**Figure 3**

**Negative Affect**

*Model 2.a*

- **Indirect effect** $a \times b = 0.0001$, $p = \text{n.s.}$
  - 95%CI [0.0000, 0.0004]

- **Direct effect** $c' = 0.0005$, $p = 0.011$
  - 95%CI [0.0001, 0.0009]

- **Total effect** $c = 0.0006$, $p = 0.002$
  - 95%CI [0.0002, 0.0010]

- **Estrogen** to **Hippocampal Volume** to **Negative Affect**
  - $a = 0.000$, $p = 0.067$
    - 95%CI [0.0000, 0.0000]
  - $b = 11.6847$, $p = 0.269$
    - 95%CI [-9.6781, 33.0475]

*Model 2.b*

- **Indirect effect** $a \times b = 0.0004$, $p = \text{n.s.}$
  - 95%CI [-0.0003, 0.0018]

- **Direct effect** $c' = 0.0026$, $p = 0.019$
  - 95%CI [0.0005, 0.0047]

- **Total effect** $c = 0.0030$, $p = 0.003$
  - 95%CI [0.0011, 0.0049]

- **Estradiol** to **Hippocampal Volume** to **Negative Affect**
  - $a = 0.000$, $p = 0.025$
    - 95%CI [0.0000, 0.0001]
  - $b = 9.9432$, $p = 0.374$
    - 95%CI [-12.7566, 32.6429]
Mediation analysis of hormonal levels, negative affect, and hippocampal volumes. Path a is the linear effect of the hormonal levels (independent variable) on hippocampal volume. Path b is the effect of hippocampal volume (mediator) on negative affect (outcome variable). The indirect effect a*b measures the amount of mediation, and the direct effect c’ is the effect of the hormonal levels on negative affect after controlling for hippocampal volume. The total effect c is the sum of direct and indirect effects. All paths’ estimates are depicted as regression coefficients, respective p-values and 95% confidence interval (95%CI). Significant results are indicated in bold. n.s. = non-significant. Hormone icon pictogram, source: iStock. Licensed under the standard license.

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