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Ultra-high-field 7T MRI reveals changes in human medial temporal lobe volume in female adults during menstrual cycle

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Ovarian hormones have substantial effects on the brain, and early menopause has been associated with increased risk of accelerated brain aging and dementia later in life. However, the impact of ovarian hormone fluctuations on brain structure earlier in life is less understood. Here we show that ovarian hormone fluctuations shape structural brain plasticity during the reproductive years. We use longitudinal ultra-high field neuroimaging across the menstrual cycle to map the morphology of medial temporal lobe subregions in 27 participants. Controlling for water content and blood flow, our findings reveal positive associations between estradiol and parahippocampal cortex volume, progesterone and subiculum and perirhinal area 35 volumes, and an estradiol*progesterone interaction with CA1 volume. This research offers a blueprint for future studies on the shared dynamics of the brain and ovarian function and a fundamental stepping stone towards developing sex-specific strategies to improve brain health and mental health.

Ovarian hormones are powerful modulators of neuroplasticity, with animal research offering robust evidence of endocrine regulation of brain morphology on a rapid timescale[1]. In the timescale of hours to days, rodent and non-human primate studies have demonstrated that estradiol and progesterone elicit modulatory effects on cell proliferation[1], dendritic spine and synapse density[2–5], mitochondrial and synaptic health[6,7], synaptic sprouting and axon growth[8–10], and myelination[11], suggesting a pivotal role of ovarian hormones in brain structural organization. In humans, the menstrual cycle provides an opportunity to study how endogenous fluctuations in hormones may transiently influence the brain, as estradiol levels increase eightfold and progesterone levels 80-fold over a period of ~25–32 days[12]. Although a growing number of menstrual cycle studies suggest that ovarian hormone fluctuations do influence brain function and behavior in humans[13–15], it remains less clear how endocrine factors may shape brain structure following the rhythmic nature of the menstrual cycle, and the implications this would have for human adult neuroplasticity.

In this context, the hippocampus is a key region shown to display a remarkable degree of neuroplasticity during the reproductive years, such as during pregnancy[16–20] and during the menstrual cycle[21–23]. The hippocampus is also implicated in emotional regulation and cognition[1,24,25], domains that are susceptible to cycle-dependent fluctuations[14,15]. The hippocampus and extended medial temporal lobe (MTL) are rich in estradiol and progesterone receptors[26,27], and previous studies suggest that estradiol-dominant menstrual cycle phases are associated with greater hippocampal volume[21–23]. Findings have been inconsistent, however, as menstrual cycle studies typically only assess two timepoints and do not directly measure ovarian hormone levels,

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rather using cycle phase as a proxy for hormone states[22,23]. Recently, a different approach using dense-sampling of hormones and brain across the menstrual cycle has been developed. Compared to sparse-sampling approaches, which typically just measure cycle phases corresponding to peak/trough hormone concentrations, dense-sampling designs assess many menstrual cycle stages to better capture dynamic interactions between the endocrine and nervous systems. For example, in a single-subject pilot study[28], we observed that subtle gray matter density changes in the human hippocampus paralleled daily fluctuations in endogenous estradiol levels, while intrinsic functional connectivity changes were associated with endogenous progesterone levels across the menstrual cycle[29], a dynamic pattern that would have been overlooked with a sparse-sampling approach. The '28andMe' project[30] has also elegantly shown that dense-sampling ($n = 1$ female participant, sampled 30 days across the full menstrual cycle, 30 days on a hormonal contraceptive regimen) can uncover unique brain–hormone interactions in brain function[13,31,32] and structure[33] that would otherwise be overlooked, setting the stage for additional studies in larger cohorts. Thus, the hippocampus and surrounding MTL are promising targets for cycle-related hormonal modulation of structural brain plasticity, but study designs require densely sampled hormone and neuroimaging data over the timescale of the entire menstrual cycle to best capture intra- and interindividual variability in both cycle variation and brain structure.

Moreover, although most human magnetic resonance imaging (MRI) studies treat the hippocampus as a homogeneous structure, recent advances in neuroimaging allow for more precise delineation of neuroanatomical subregions of the hippocampus and MTL in vivo in humans[34–36]. This specificity is important given the unique cytoarchitecture, chemoarchitecture and circuity of MTL subregions[36–38] that differentially contribute to aging and disease[39,40]. For example, previous studies have shown that visual memory is more dependent on the perirhinal cortex, that spatial memory is more dependent on the parahippocampal cortex, that the subiculum plays an important role in mediating hippocampal–cortical communication, and that the hippocampus is crucial for both semantic memory and episodic memory but that lesion location within the hippocampus uniquely impairs these memory types (reviewed in refs. 41,42). Subregion-specific architecture and circuitry, alongside potential differences in hormone receptor densities[43], suggest that hormone-modulated volumetric changes may manifest differently across the MTL complex, such as has been recently shown in women with early oophorectomy[44]. Supporting evidence for the influence of ovarian hormone fluctuations on subregions mainly stems from animal work, with particular emphasis on the cornu ammonis 1 (CA1), a subregion critical for memory integration[45] and in which neuronal loss has been associated with Alzheimer's disease in an all-male human study[46]. Estradiol administration enhances synaptogenesis and spine density in CA1 neurons of ovariectomized adult rodents[2,4,47] and ovariectomized young and aged non-human primates[48], whereas progesterone inhibits this effect. A study in ovariohysterectomized young-to-middle-aged primates found that estradiol treatment increases pre- and post-synaptic proteins in CA1, whereas combined estradiol and progesterone decreases these synaptic proteins[49]. As women have a greater risk of developing Alzheimer's disease relative to men[50], an additional region of interest is perirhinal area 35, a subregion in which atrophy has been associated with cognitive decline as well as early stages of dementia in older adult mixed-sex studies that either controlled[51,52] or did not control for sex[53–55]. Area 35, corresponding to the transentorhinal region and medial perirhinal cortex[34,55], has thus far received little attention regarding endogenous ovarian hormone fluctuations. This constitutes a critical knowledge gap given the relevance of this subregion to dementia progression and that changes in hormone status have been proposed to underlie sex differences in Alzheimer's disease pathology[56–58]. Hence, investigating the influence of subtle hormone fluctuations on CA1 and area 35 volume is probably highly relevant for a mechanistic understanding of female-specific cognitive decline risk.

Table 1 | Baseline demographics

Characteristic	Mean	S.d.	Range
Age (years)	25.33	3.64	19–34
Body mass index (kg m^{-2})	22.37	3.64	18.8–29.0
Menstrual cycle length (days)	29.04	2.62	25–34
Ethnicity	N=27, 100% European/German without self-reported migration background or foreigner status		

Initial evidence for hormone-modulated changes across human MTL subregions has been observed in a recent single-subject study using 3-T MRI, in which the authors observed associations between daily ovarian hormone levels and MTL complex volumes[33]. No study has yet applied a high-density sampling protocol to test whether consistent patterns of hormone–volume associations at the subregion level can be identified in the human hippocampus and surrounding MTL in multiple participants across the menstrual cycle. To shed further light on hormone-associated hippocampal and MTL changes in the female human brain during the reproductive years, we provide a densely sampled and detailed ultra-high field neuroimaging dataset in 27 healthy participants who underwent 7-T MRI scanning during six menstrual cycle phases: menstrual, pre-ovulatory, ovulation, post-ovulatory, mid-luteal and premenstrual. We utilized the Automated Segmentation of Hippocampal Subfields software (ASHS[35]), which allows for a sensitive approach to individual differences in MTL subregion morphology[59]. Notably, the chosen Magdeburg Young Adult 7T Atlas[34] leverages information on anatomical variability, resulting in a more accurate delineation of the boundary between the CA1 and subiculum, CA3 and dentate gyrus, as well as the parahippocampal cortex, and further segmentation of the perirhinal region into areas 35 and 36. These advantages, alongside 7-T MRI images with small slice thickness and high in-plane resolution, allow for better delineation and segmentation of the MTL subregions. We also developed a systematic protocol for rigorous cycle phase characterization to overcome the inaccuracy of menstrual cycle monitoring, a limitation of previous work in this field[60–62]. Based on our pilot study[28], we hypothesized that cycle-related increases in estradiol levels would be associated with increases in whole hippocampus volume. Within the subregions, based on the above-mentioned animal literature, we hypothesized that estradiol levels would be positively associated with perirhinal area 35 volume, and that there would be an interaction between estradiol and progesterone levels in CA1 volume. Other subregion volumes (CA2, CA3, subiculum, dentate gyrus, area 36, entorhinal and parahippocampal cortices) were assessed in an exploratory fashion. Given the essential role of the hippocampus and MTL in adult neuroplasticity, these findings may contribute to a better understanding of how endocrine factors shape healthy adult brain dynamics during the reproductive years as well as inform more individualized strategies for neuroimaging the female brain.

Results

Monitoring

All baseline demographics are reported in Table 1. Participants were of reproductive age, with healthy body mass index (BMI) and regular menstrual cycle length. Endogenous ovarian hormone values, subregion volumes and whole hippocampus volume (sum of CA1, CA2, CA3, subiculum, dentate gyrus and remaining tail) were within the expected ranges (Fig. 1 and Table 2). For further analyses, hormone values were log-transformed and bilateral subregion volumes were adjusted for total brain volume, and statistical significance was accepted at a Benjamini–Hochberg false detection rate (FDR) corrected threshold of $q < 0.05$ (see Methods for additional monitoring and statistical details).

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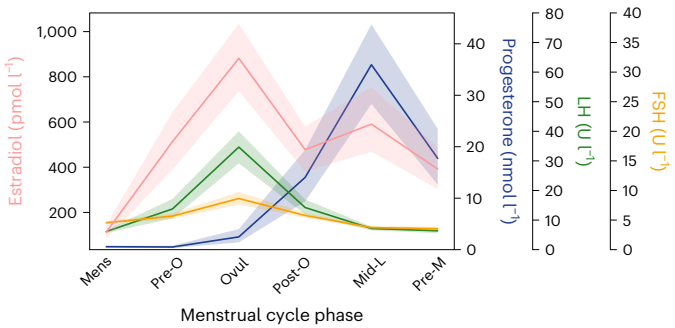

Fig. 1 | Changes in endogenous levels of estradiol, progesterone, LH and FSH across the menstrual cycle. Lines and shaded areas represent mean values with 95% CIs for hormone levels at Mens, Pre-O, Ovul, Post-O, Mid-L and Pre-M timepoints. $N = 27$.

Table 2 | Descriptive statistics for each brain region volume

Brain region	Mean (mm^3)	S.d. (mm^3)	Range (mm^3)
Total brain volume	1,130,246.38	83,996.77	412,000.00
Whole hippocampus	5,295.36	381.71	2,322.00
CA1	1,399.73	132.36	586.75
CA2	86.49	21.22	101.25
CA3	257.00	37.31	167.00
Dentate gyrus	905.39	117.94	534.00
Subiculum	2,043.41	146.34	739.25
Entorhinal cortex	1,151.98	165.34	852.50
Area 35	752.80	104.35	585.25
Area 36	4,046.27	543.87	1,959.25
Parahippocampal cortex	660.92	97.75	540.00

Whole hippocampus is the sum of cornu ammonis CA1, CA2, CA3, subiculum, dentate gyrus and the remaining tail. $N = 27$.

Control analyses

Previous work in the field has been critiqued for not taking into account potential hormone-related water shifts or cerebral blood flow (CBF) changes in the brain, which could be misconstrued as hormone-related brain-volume changes. Although the Magdeburg Young Adult 7T Atlas does exclude the alveus, fimbria, cerebrospinal fluid (CSF) and blood vessels, we additionally assessed CSF and CBF changes in the hippocampus and did not observe statistically significant associations with estradiol (CSF: $\beta = 4.28$, 95% CI = −14 to 6, random effects s.d. = 22.62, $P = 0.402$; CBF: $\beta = 1.23$, 95% CI = −3 to 1, random effects s.d. = 4.19, $P = 0.195$) or progesterone (CSF: $\beta = 3.67$, 95% CI = −1 to 8, random effects s.d. = 22.43, $P = 0.134$; CBF: $\beta = 0.06$, 95% CI = −1 to 1, random effects s.d. = 4.22, $P = 0.899$), giving further confidence that the following results were not erroneously driven by these factors.

The bilateral precentral gyrus was identified as a control region outside the MTL, as this brain region is not reported to be strongly influenced by fluctuations in ovarian hormones. We did not observe statistically significant associations between precentral gyrus volume with estradiol nor with progesterone (estradiol: $\beta = 3{,}477.33$, 95% CI = −747 to 702, random effects s.d. = 9,415.84, $P = 0.106$; progesterone: $\beta = -161.23$, 95% CI = −2,232 to 1,910, random effects s.d. = 9,564.48, $P = 0.878$).

Ovarian hormones and MTL volumes across the menstrual cycle

As hypothesized, linear mixed-effects modeling showed positive associations between estradiol levels and whole hippocampus volume ($\beta = 108.26$, 95% CI = 27 to 190, random effects s.d. = 174.47, $P = 0.009$). In MTL subregions, addition of the estradiol*progesterone interaction to the model significantly improved model fit only for CA1 (Fig. 2a; $\chi^2(1) = 7.691$, $P = 0.006$). Estradiol was positively associated with CA1 volume, progesterone was negatively associated with CA1 volume, and we observed a significant interaction of estradiol and progesterone with CA1 volume (estradiol: $\beta = 42.87$, 95% CI = 21 to 65, $P < 0.001$; progesterone: $\beta = -150.02$, 95% CI = −249 to −51, $P = 0.003$; interaction: $\beta = 53.06$, 95% CI = 16 to 90, random effects s.d. = 44.03, $P = 0.005$), such that at higher progesterone levels, the positive effect of estradiol on CA1 volume was attenuated. Progesterone was positively associated with subiculum volume (Fig. 2b) and with area 35 volume (Fig. 2c) (subiculum: $\beta = 13.12$, 95% CI = 4 to 22, random effects s.d. = 43.29, $P = 0.006$; area 35: $\beta = 11.98$, 95% CI = 2 to 21, random effects s.d. = 44.01, $P = 0.014$). Finally, estradiol was positively associated with parahippocampal cortex volume (Fig. 2d; $\beta = 24.33$, 95% CI = 10 to 39, random effects s.d. = 32.48, $P = 0.001$). All four subregions showed significant changes in volume over the six cycle phase timepoints (Fig. 2, column 2), but such cycle phase effects did not survive correction for multiple comparisons in the whole hippocampus or subiculum. We did not observe any significant relationship between hormones and CA2, CA3, dentate gyrus, entorhinal cortex or area 36 volumes ($0.196 \leq P \leq 0.884$) (Fig. 1).

Discussion

In this Article we have combined dense-sampling of hormones and brain with individually derived high-resolution MTL segmentation analysis to demonstrate how estradiol and progesterone fluctuations affect key memory regions, such as the MTL and hippocampal subregion volumes. Parahippocampal cortex, area 35, subiculum and CA1 volumes showed significant changes in association with hormone fluctuations across the menstrual cycle. More specifically, estradiol levels were positively associated with parahippocampal cortex volume, and progesterone levels were positively associated with subiculum and area 35 volume. We also observed an estradiol*progesterone level interaction with CA1 volume. The observed volumetric changes and their unique associations with ovarian hormones, especially progesterone, were concealed when analyzing the hippocampus as a whole, a potential limitation for neuroimaging studies that traditionally assess the hippocampus as a single homogeneous structure. We provided further confidence that the observed effects do not erroneously stem from extra-neuronal factors, such as hormone-induced changes in regional CBF or CSF expansion, as we did not find associations between these factors and ovarian hormone levels. Finally, we also did not observe any associations between ovarian hormone levels and brain volume in our control region, the precentral gyrus. Taken together, our results suggest that ovarian hormones rapidly alter structural brain plasticity during the reproductive years and that hippocampal and MTL subregions may be differentially sensitive to hormones.

We were especially interested in CA1 and area 35 given previously observed selective patterns of neuronal vulnerability to memory impairment in CA1 in an adult all-male study[46] and area 35 in older adult mixed-sex studies[51–55], as women are more likely to suffer from cognitive impairment when ovarian hormones rapidly fluctuate, such as during perimenopause[57,58,63]. The hypothesized interaction in CA1 is in line with previous animal work, showing that estradiol enhances synaptogenesis, spine density and synaptic protein levels in CA1, while subsequent increases in progesterone seem to inhibit this effect[2,4,7,47–49]. Moreover, progesterone administration decreases dendritic spines in ovariectomized adult rodent CA1 neurons, an effect that can be inhibited with a progesterone receptor antagonist[2]. Although studies in humans are limited and larger brain volume does not necessarily imply better function, we do know that CA1 plays a distinct functional role in memory integration and inference[45]. Clinical studies in post-menopausal women have also shown that estrogen replacement is associated with maintaining cognitive function in older age, and progesterone may counteract

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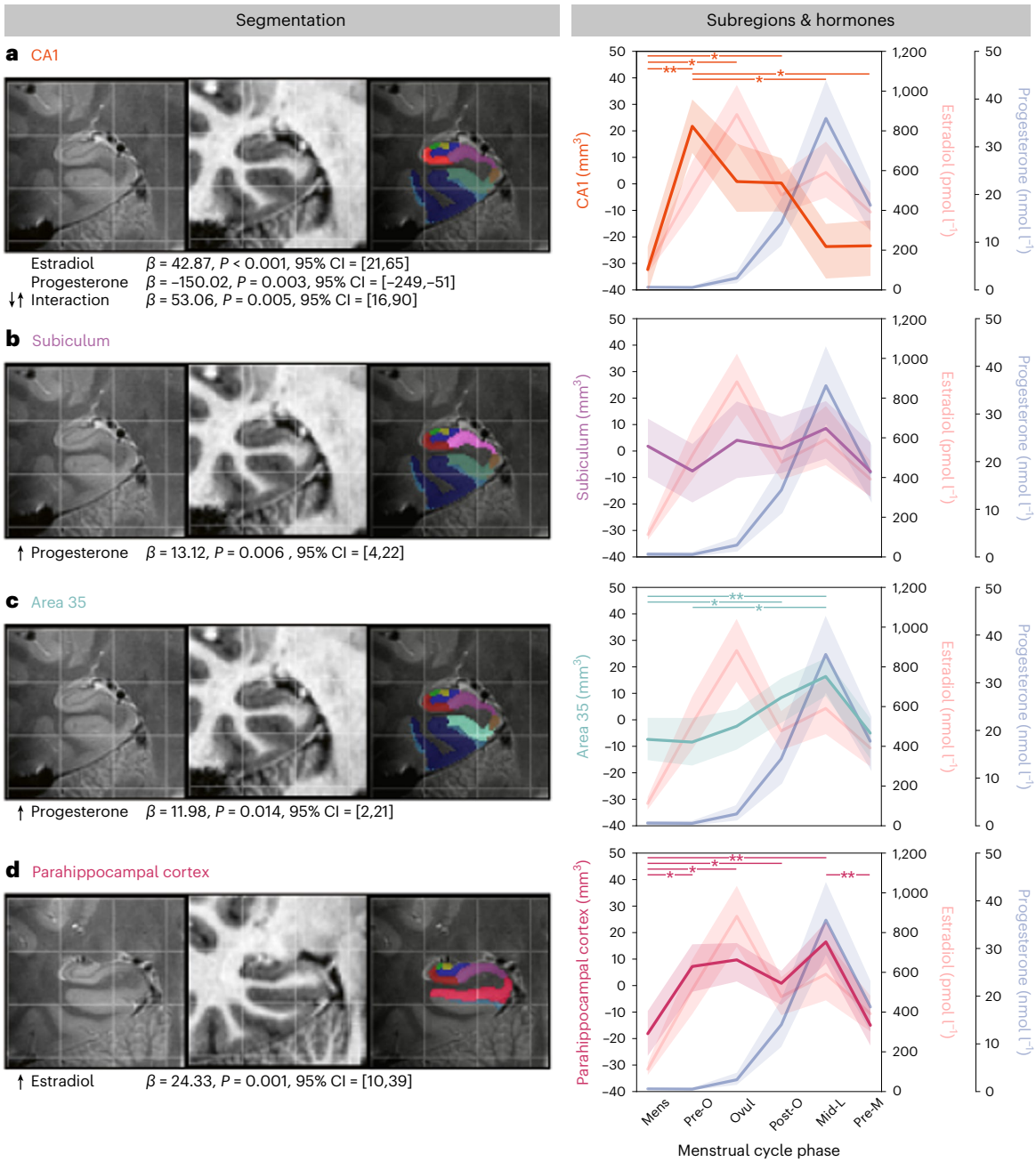

Fig. 2 | Changes in MTL volume associated with ovarian hormones across the menstrual cycle. a–d, Column 1: example T2-weighted image, T1-weighted image and MTL segmentation for CA1 (**a**), subiculum (**b**), perirhinal area 35 (**c**) and parahippocampal cortex (**d**). Column 2: after segmentation, unique associations between ovarian hormones and MTL regions across the menstrual cycle (Mens, menstrual; Pre-O, pre-ovulatory; Ovul, ovulation; Post-O, post-ovulatory; Mid-L, mid-luteal; Pre-M, premenstrual). Linear mixed-effects model statistics are shown (with uncertainty intervals (equal-tailed) and *P* values (two-tailed) computed using a Wald *t*-distribution approximation, and corrected for multiple comparisons using the Benjamini–Hochberg procedure at an FDR-corrected threshold of $q < 0.05$). The center of the error band represents the mean, with the shaded area representing +95% CI based on the bootstrap distribution for hormone levels or subregion volumes. Asterisks refer to statistically significant changes (corrected for multiple comparisons) in volume over timepoints. Details are as follows. **a**, CA1: Mens versus Pre-O, $P = 0.001$; Mens versus Post-O, $P = 0.006$; Pre-O versus Mid-L, $P = 0.022$; Pre-O versus Pre-M, $P = 0.025$. **c**, Area 35: Mens versus Mid-L, $P = 0.004$; Pre-O versus Mid-L, $P = 0.007$; Mens versus Post-O, $P = 0.025$. **d**, Parahippocampal cortex: Mens versus Mid-L, $P = 0.0001$; Mid-L versus Pre-M, $P = 0.005$; Mens versus Pre-O, $P = 0.007$; Mens versus Post-O, $P = 0.02$; Mens versus Pre-O, $P = 0.042$. $^*P < 0.05$, $^{**}P < 0.005$. $N = 27$.

the benefit of estradiol's cognitive enhancing effect[64–66]. We note, however, that although most hormone replacement therapy studies suggest that estrogen replacement has a beneficial effect on cognition, literature on progesterone is less consistent, with some studies finding positive effects of progesterone[67] and other studies finding little, no or negative effects of progesterone on cognitive function[64–66,68]. Thus, our CA1 findings are consistent with previous animal and clinical work suggesting a proliferative effect of estradiol and suppressive effect of progesterone on synaptic plasticity in CA1, a region critical for cognitive processes. Furthermore, as reviewed in the discussion of our next finding, progesterone may have differing effects on brain regions relevant to cognition.

We also observed a positive association between progesterone levels and area 35 volume, which corresponds to the transentorhinal

region and medial perirhinal cortex[34,55]. This region is clinically relevant with regard to aging and disease, with previous work showing that neurodegeneration and atrophy in this region is associated with early stages of dementia and cognitive decline in mixed-sex studies[51–55]. Given the increasing focus on the interplay between endogenous hormone fluctuations, the MTL and the disproportionate risk for Alzheimer's disease in women[50,58,63], we hypothesized that estradiol fluctuations would be associated with area 35 volumetric changes. The majority of related literature on hippocampal structural plasticity has thus far focused on estradiol relative to progesterone, and the CA1 and dentate gyrus relative to other MTL regions. With the observed progesterone association in this study, additional emphasis on progesterone in future research is warranted given that its levels change ~80-fold over the menstrual cycle[12], and that both our current findings and previous work[33] show a complex role of progesterone on MTL subregion volumes. We note that, although the single-subject study[33] also observed both positive and negative associations between progesterone and subregion volumes, our observed progesterone findings occurred in different areas of the MTL, whereas the other study observed associations with CA2/3, perirhinal, entorhinal and parahippocampal cortex volumes. These differences may be partially driven by differences in the study design and methodology, such as the number of participants, scanner strength, segmentation atlas used and differences in timepoints. Comparing effect sizes in our current study to previous work remains difficult, as the mentioned study[33] is the only other study to measure changes in MTL subregion volume across the menstrual cycle, and differences in study design and methodology (especially 3-T versus 7-T) make direct comparison challenging (although we do note that in ref. 33 ~8% volume change in CA1 from menses to pre-ovulatory stages was observed, and we observed a comparable 5%). We therefore encourage replication of the current findings in future studies.

Beyond the hypothesized regions, we also observed positive associations between estradiol and parahippocampal cortex volume, and progesterone and subiculum volume. Although the effect of menstrual cycle timepoint did not survive correction for multiple comparisons in the subiculum, changes in subiculum and parahippocampal cortex volumes have been observed during pregnancy[17,18] and after surgical menopause[69], both of which are times of more extreme changes in ovarian hormones. The subiculum has also been previously shown to display sex-specific volumetric changes in healthy cognitive aging[39] and plays an important role in mediating hippocampal–cortical interactions[42], which are critically involved in cognitive function and emotional regulation, processes influenced by menstrual cycle phase[14,15]. Although we highlight the relevant strengths of the methods used in this Article (for example, an atlas with the distinction of area 35 and a better boundary of subiculum and parahippocampal cortex[34]), future research is required to replicate the current findings in these subregions, as our study clearly demonstrates complex interactions between ovarian hormones and MTL structure as a first step in understanding hormone-induced modulation of brain plasticity at the subregion level.

Although the nature of our MRI study cannot directly evaluate the physiological processes underlying MTL morphology changes, we can speculate on potential hormone-induced molecular and cellular mechanisms that may contribute to mesoscopic changes in regional brain volume. Animal research lends proof that ovarian hormones serve as critical components of cell survival and plasticity, where possible mechanisms at the microanatomical level could include cell proliferation and microglial activation[1,70], dendritic spine and synapse density[2–5], mitochondrial and synaptic health[6,7], synaptic sprouting and axon growth[8–10], and myelination[11], which can occur in a matter of hours to days. These hormone-induced actions can occur through activation of classical estrogen (ERα and ERβ) and progesterone receptors (PRs), which are densely found in the MTL complex[26,27]. For example, previous work has shown that ER antagonists and agonists can respectively decrease and increase cell proliferation in the dentate gyrus of ovariectomized adult rodents[71,72]. We note that we and others[33,49] did not, however, observe volumetric changes in this region across the menstrual cycle, suggesting that the MTL changes we observe may be more driven by synaptic plasticity and remodeling in regions such as the CA1 as opposed to neurogenesis in the dentate gyrus. Indeed, within the hippocampal complex, these three classical receptors appear most prominently in the CA1[43], where estradiol administration and ER agonists increase synaptic proteins[49,73], ER antagonists decrease synaptic proteins[74], and progesterone administration and PR antagonists respectively decrease and increase dendritic spines[2,4]. These modulatory effects were not as prominent in the dentate gyrus and other subregions as in CA1 (refs. 43,49,73,74). Given that previous work has shown that expression of such receptors varies across MTL subregions and across the estrous cycle[43], this may provide a mechanism for hormone-related changes in molecular and cellular processes that contribute to MTL subregion-specific volumetric changes observed across the menstrual cycle. However, given differences in scale, these synaptic-level mechanisms cannot fully account for the observed changes in subregion volumes. Our study thus encourages future investigation of the specific biological microstructural mechanisms underlying the observed short-term dynamics of human MTL subregion morphology during times of ovarian hormone fluctuations.

Although the rigorous cycle monitoring protocol and individually derived MTL segmentation analyses serve as strengths of this sufficiently powered longitudinal study, several limitations should be acknowledged. We recruited a healthy population to reflect how endogenous endocrine factors regulate healthy adult brain structural plasticity in the female reproductive years. Most of the reviewed background literature, however, is based on studies using exogenous hormone manipulation and/or populations with a different reproductive status, such as ovariectomized animals or post-menopausal female adults. Reproductive phase (for example, menstrual cycle versus menopause), type of hormone change (for example, endogenous fluctuations versus exogenous manipulation) and timing (for example, years since menopause) can all influence how the brain reacts to changes in ovarian hormone status[1,68,75,76]. For example, the benefit of hormone therapy on cognitive health and potentially hippocampal plasticity depends on being initiated near the time of cessation of ovarian function[76]. Therefore, future studies should test whether the observed menstrual-cycle hormone-induced changes in MTL subregions are further exacerbated during times of more profound neuroendocrine change, such as during perimenopause or early oophorectomy, and whether there are behavioral consequences for patient populations. Second, selection bias could emerge if participation is limited by hesitation to participate in multiple neuroimaging sessions and/or rigorous menstrual-cycle monitoring procedures. Given the longitudinal design of the study and the multiple assessments, this cannot be ruled out. The strengths of this within-subject design include higher statistical power and better control of participants' characteristics or individual differences, such as head motion affecting the individual study outcomes. We also acknowledge the possibility of attrition bias, which is inherent to protocols with multiple follow-up appointments. By careful randomization and controlling for socio-demographic and lifestyle factors, such as education level and perceived stress, we aimed to limit the impact of such bias. Due to the majority of the population in Saxony being of German ethnicity, our sample is 100% European/German. As results from one population may not extrapolate well to another, we need further research to expand neuroimaging studies to enhance representation of multiple populations, and prioritize samples of non-European descent. Finally, we studied an all-female sample. This limits the generalizability of our findings to the male population, but provides relevant information that has been lacking in the neuroscience literature.

Furthermore, recent work does suggest that early changes in neuropsychiatric and neurodegenerative disease are better detected in smaller subregions of the MTL rather than with whole-structure

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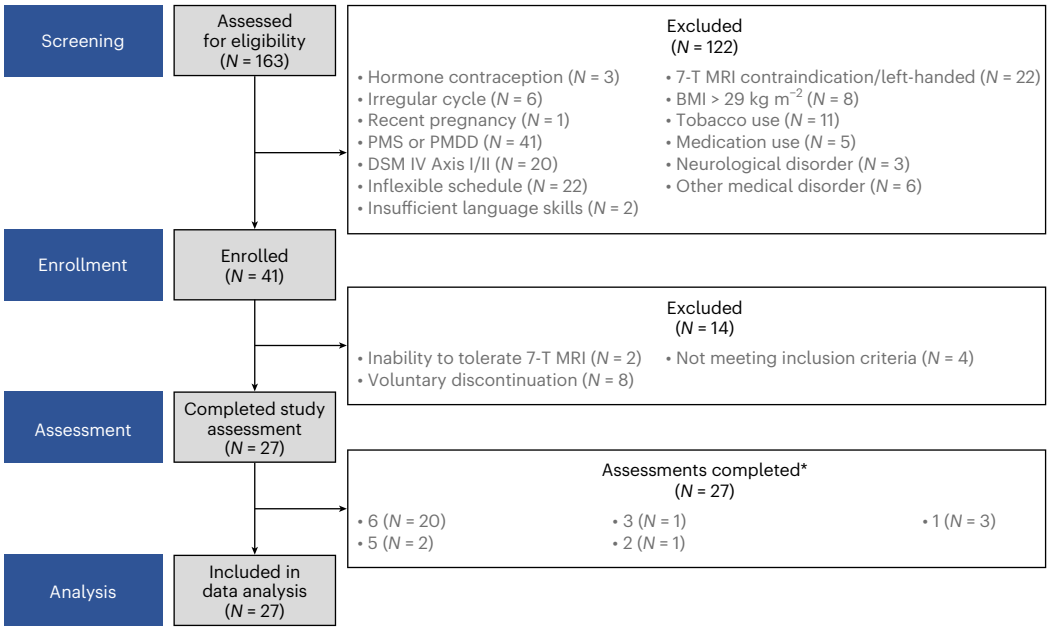

Fig. 3 | Flow diagram for the study. Note that participants may fall into more than one screening exclusion category. *Of the assessed participants, 20 completed all six assessments, two completed five assessments, one completed three assessments, one completed two assessments, and three completed one assessment (dropout reasons: five due to scheduling conflicts, one used the emergency contraceptive pill, one got an MRI-incompatible retainer). PMS, premenstrual syndrome; PMDD, premenstrual dysphoric disorder.

analysis[51–53,77], and women are at increased risk for such disorders following ovarian hormone fluctuations, such as depressive disorders[78–81], multiple sclerosis[82–84], dementia[57,58] and other autoimmune disorders[85], during the premenstrual phase[78,79,82,85], postpartum period[78,80,83] and following perimenopause[57,58,78,81,84]. Given the essential role of the MTL in adult synaptic plasticity and the differential contributions of MTL subregions to cognitive functions[45,86–89], this information may be particularly relevant for plasticity-related disorders such as depression and dementia, which women are at greater risk of developing[50,90,91]. Furthermore, although we aimed to assess endogenous ovarian hormones, future studies should also consider having a control group in which endogenous estradiol and progesterone fluctuations are effectively clamped, such as a hormonal contraception group. Finally, while our analyses focused on the hippocampal and MTL complex, ovarian hormones have widespread effects on many brain areas that work in tandem. Subregions have distinct connectivity profiles[37], and future studies should extend the focus to other brain areas to capture a broader network understanding. Given that we saw unique hormone interactions with the CA1 and subiculum, and that hippocampal projections to the medial prefrontal cortex (PFC) originate primarily in the CA1 and subiculum[92], we suggest investigation of hormone-modulated changes in structural and functional connectivity between these subregions and the PFC. The PFC also densely expresses hormone receptors[93] and displays hormone-induced structural plasticity[3,6,7], with the hippocampal–prefrontal pathway implicated in cognitive and emotional processes[42,92].

In conclusion, our study incorporated several factors to account for external validity (for example, a longitudinal design that enabled the examination of within-subject changes over time, and the utilization of advanced neuroimaging techniques, such as ultra-high field imaging and individually derived segmentation analysis), contributing to more robust and reliable findings. Most critically, however, this study addresses the considerable gap of missing female data in the neurosciences, thus providing a substantial contribution to improving the generalizability of MR-based findings of structural volumetric changes and neuroplasticity in the adult human brain. Despite decades of scientific evidence for dynamic interactions between the endocrine and nervous systems, neuroscientific research has largely ignored how endogenous ovarian hormone fluctuations influence human female brain structural plasticity. The current underrepresentation of female samples[61,94,95] and female-health variables, such as ovarian hormones, directly limits opportunities for basic scientific discovery and the diversity of human brain health. Our findings suggest that the MTL region has a remarkable degree of plasticity in response to subtle hormone changes during the reproductive years, and that these changes are detectable at the subregion level. Using ultra-high field neuroimaging, this study shows how endogenous hormone fluctuations rapidly and transiently alter volume across the MTL complex in within-subject measurements in multiple participants. We demonstrate the feasibility of a longitudinal MRI design for creating dynamic and personalized maps of the human brain to inform more individualized strategies for neuroimaging the MTL, which may ultimately lead to better understanding of the manifestation and treatment of hormone-related neuropsychiatric and neurodegenerative diseases.

Methods

Participants

Between 15 May 2015 and 21 February 2019, 163 participants were screened, of which 43 could be enrolled in this neuroimaging study in Leipzig (Fig. 3). Participants were financially compensated for study participation. Eligible individuals were female, right-handed, 18–35 years old, with a BMI of 18.5–29 kg m^{-2}, and without any neurological or psychiatric illness (as confirmed with a structured clinical interview and consultation from a licensed psychiatrist, J.S.)[62,96]. Exclusion criteria were prescription medication or supplement use, tobacco use, positive drug or pregnancy tests, current use or use of hormonal contraceptives within the past six months, current or past hormone therapy, or having been pregnant, postpartum, breastfeeding, or had an abortion within one year of the study. Participants were screened and excluded for DSM-IV axis I disorders and axis II disorders, as well as for the presence of premenstrual mood symptoms using the Premenstrual Symptoms Screening Tool (PSST)[97]. All participants provided written informed consent after all procedures were fully explained. Forty-one participants were enrolled, of whom two were excluded due

to inability to tolerate the 7-T MRI scan, eight voluntarily discontinued because of the time demands of the study, and four were excluded due to irregular cycles, irregularities in bloodwork, or emergency contraceptive pill use after enrollment (included participants $N = 27$). Of the included participants, 20 completed all six timepoints, two completed five timepoints, one completed three timepoints, one completed two timepoints, and three completed one timepoint (dropout reasons: five due to scheduling conflicts, one used the emergency contraceptive pill, one got an MRI-incompatible retainer), for a total of 138 assessments. The study was conducted in accordance with the Declaration of Helsinki. The Ethical Committee at the Medical Faculty of Leipzig University approved the study, protocol and informed consent forms (#077-11-07032011). The study has been pre-registered at the Open Science Framework (https://osf.io/8mk74/) and is registered on the German Clinical Trials Register (DRKS00030983).

Assessment timing

Participants had a documented history of regular menstrual cycles. In this longitudinal study, assessments occurred during six cycle phases: menstrual (<5 days menses onset), pre-ovulatory (≤2 days before ovulation), ovulation (≤24 h of ovulation), post-ovulatory (≤2 days after ovulation), mid-luteal (6–8 days after ovulation) and premenstrual (≤3 days next menses onset). We developed a systematic protocol for rigorous menstrual cycle monitoring and characterization to determine cycle phase timing as follows. After enrollment, participants used an online application (https://www.mynfp.de) to record daily vaginal basal body temperature, menses information, and cycle day and length information[62]. Participants recorded menstrual cycle information for at least one full cycle before participating in the six assessments, to provide more information on cycle regularity and length. To determine ovulatory timing, participants underwent multiple vaginal ultrasounds to track growing follicle and detect ovulation, completed luteinizing hormone (LH) urine tests throughout the ovulatory week, and consulted with a gynecologist[62]. To determine premenstrual timing, we estimated the luteal phase to last ~14 days, supplemented with information from previous menstrual cycle lengths, ovulatory timing and consultation with a gynecologist. The first assessment phase was randomized across participants, and all other assessments took place in remaining chronological order (menstrual, pre-ovulatory, ovulation, post-ovulatory, mid-luteal, premenstrual). At every vaginal ultrasound and assessment visit, hormone levels (estradiol, progesterone, LH, follicle-stimulating hormone (FSH)) were assessed in blood and used to confirm the current cycle phase (based on reference ranges provided by ref. 98 and ECLIA, Cobas:Roche) (Fig. 1).

Blood measurements

Serum from fasting-blood samples was collected at every ultrasound and assessment day visit to measure hormones, confirm cycle phase, ensure physical health, and exclude pregnancy or recent drug-intake. Serum was immediately delivered to the hospital laboratory and kept at 5 °C until assayed within 24 h (ref. 62). Estradiol and progesterone concentrations were determined using high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), and follicle-stimulating hormone and luteinizing hormone concentrations were determined using electrochemiluminescence immunoassay (ECLIA; Roche). Of the 138 assessments, estradiol values for two assessments and progesterone values for one assessment were not included due to pre-analytical error.

7-T MRI acquisition

Anatomical MRI scans were acquired at the Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, using a Siemens Magnetom 7-T system (Siemens Healthineers) and 32-channel head array coil (NOVA Medical), matched for time of day and without caffeine intake. We acquired high-resolution whole-brain T1-weighted images using an MP2RAGE protocol[99] (repetition time (TR) = 5,000 ms; inversion time (TI) 1/2 = 900/2,750 ms; echo time (TE) = 2.45 ms; image matrix, 320 × 320 × 240; voxel size, 0.7 mm × 0.7 mm × 0.7 mm; flip angle 1/2 = 5°/3°; parallel imaging using GRAPPA with acceleration factor = 2). We acquired T2-weighted imaging slabs perpendicular to the anterior–posterior axis of the hippocampus using a Turbo-Spin Echo Sequence (TR = 16,000 ms; TE = 14 ms; image matrix, 384 × 384; 50 slices; voxel size, 0.5 mm × 0.5 mm × 1 mm; refocusing flip angle = 120°; turbo factor = 8; parallel imaging using GRAPPA with acceleration factor = 2).

To minimize motion during the scan, the head, neck and shoulders were secured with foam cushions and the legs were secured with sandbag cushions on the sides. Participants were covered with a blanket to minimize motion of arms and legs, and they were verbally instructed and continuously reminded to remain as still as possible. All incoming images were visually assessed and immediately re-run if motion was detected (this occurred in nine scanning sessions).

MTL segmentation and volumetry

Background noise removal from uniform T1-weighted MP2RAGE image volumes was done using https://github.com/JosePMarques/MP2RAGE-related-scripts. The high-resolution T1- and T2-weighted images were then submitted to the ASHS package[35] using the Magdeburg Young Adult 7T Atlas (based on 22 healthy participants; 19–32-years old; 12 females)[34], which has been shown to be more sensitive to individual differences in MTL subregion morphology compared to FreeSurfer[59]. ASHS segmentation software uses a fully automated framework at all stages (MRI pre-processing, rigid body transformation alignment of T1- and T2-weighted images, bias correction and refining, and so on), automatically segmenting the MTL in the T2-weighted MRI scans. To correct for potential motion between the T1 and T2 scans, ASHS uses both FLIRT and ANTS rigid registration algorithms, using the normalized mutual information (NMI) metric as the similarity measure. The method that yields the largest NMI value between the two modalities is used subsequently (full details are available in ref. 35). ASHS documentation, atlases and software are available at https://sites.google.com/view/ashs-dox/ and https://www.nitrc.org/projects/ashs, with technical details and reliability described further in ref. 35. We performed segmentation and bilateral volume calculations for hippocampal (CA1, CA2, CA3, subiculum, dentate gyrus) and adjacent MTL subregions (entorhinal cortex, parahippocampal cortex, perirhinal cortex (segmented into areas 35 and 36)). ITK-SNAP (v3.8) was used for quality assurance (for example, proper alignment of T1- and T2-weighted images). We did not perform manual slice number correction or segmentations, as ASHS is an automatic segmentation tool, and we wanted to best capture anatomical variability and limit idiosyncrasies and researcher degrees of freedom across the segmentation protocols. Quality assurance images were visually assessed by two raters blinded to cycle phase. To assess the reliability of segmentations, the Sørensen-Dice similarity coefficient (0.983) and intraclass correlation coefficient (0.976) were calculated across all assessments in MATLAB, suggesting robust reliability in segmentation. All code is publicly available at https://github.com/RGZsido/MTLPlasticity2022.

Total brain volume, precentral gyrus volume, CSF and CBF

Total brain volume, bilateral precentral gyrus volume and CSF were calculated using the 7-T T1-weighted images and the Segment Data module in the CAT12 toolbox of SPM12 MATLAB R2021a. Image quality was assessed using the overall image quality (image quality ratings, IQR; >91%) as well as noise (>91%), bias (>91%) and image resolution (>91%) parameters. Motion in the T1-weighted images was additionally assessed by detecting ghosting associated with head motion[100] (the ghosting ratio should not exceed three). Our values (mean ± s.d., 1.1 ± 0.6) suggest that the overall motion was negligible. Precentral gyrus volume was calculated using the Neuromorphometrics

atlas. CBF was measured using a pulsed arterial spin labeling (pASL) sequence (TR = 3,000, TI1 = 700 ms, T1S = 1,775, TI2 = 1,800 ms, TE = 13 ms, flip angle = 90°, matrix size = 64 × 64, slices = 24, field of view (FOV) = 192 × 192 mm, voxel size = 3 × 3 × 4 mm^3; labeling slab thickness = 100 mm with a gap of 22 mm, 50 pairs of label and control images), acquired on the same assessment days on a 3-T Magnetom Verio scanner (Siemens) using a 32-channel head coil. The pASL data were preprocessed using an in-house MATLAB analysis pipeline, which included co-registration to the MPRAGE image, motion correction with linear regression, normalization to MNI space using a T1-weighted MPRAGE (TR = 2,300 ms, TI = 900 ms, TE = 4.21 ms, flip angle = 9°, FOV = 256 × 256 mm, slices = 176, bandwidth = 240 Hz px^{-1}, voxel size = 1 × 1 × 1 mm^3), and smoothing with a two-dimensional (2D) spatial Gaussian filter with full-width at half-maximum of 3 mm. The CBF values used for the analysis were calculated by pairwise subtraction of labeled and control images by the perfusion model[101], as described in more detail in ref. 102.

For the CBF analysis, an anatomical region of interest (ROI) was created as a binary mask of the hippocampus using the WFU PickAtlas toolbox. The mask was resampled to a 3 × 3 × 4-mm voxel size to match the pASL images using the coregister:reslice function in SPM12. The preprocessed CBF maps were multiplied with the binary mask of the hippocampus and the average CBF value (over all voxels within the ROI) was extracted. This was done for all timepoints for each participant.

Statistical analysis

Assuming a medium effect size ($\eta^2 = 0.06$), an α coefficient of 0.05 and power of 80%, we calculated a total sample size of $N = 18$ (a priori power analysis, G*Power). In the project protocol, we stated that we aimed to include $N = 20$ healthy participants with all six timepoints. Estradiol and progesterone values were log-transformed prior to analyses. For outlier detection in brain volumes, we flagged bilateral volumes that were 3 s.d. from the mean for anatomical inspection by two raters. If, on inspection, the volume segmentation maps were unanimously deemed anatomically sound, the volumes were kept to capture reasonable anatomical variation. Of the 138 assessments, we flagged a total of four brain volumes, of which two were determined to be of poor segmentation quality (both for CA1) and were thus removed from further analyses. Bilateral subregion volumes were then adjusted for total brain volume (unstandardized residuals).

For control analyses, we performed linear mixed-effects modeling using the maximum likelihood method of the 'lmer' function in the 'lme4' R package (v3.5.2) to assess potential effects of hormones (estradiol and progesterone) on CSF, CBF and precentral gyrus volumes. For the main analyses, we used linear mixed-effects models to assess the fixed effects of hormones as well as their interaction with the whole hippocampus and each subregion volume. Inclusion of the interaction term was assessed by comparing model fits using the 'anova' function. The P values of the model parameters were calculated via Wald tests and corrected for multiple comparisons using the Benjamini–Hochberg procedure[103] controlling for FDR, and were accepted at an FDR-corrected threshold of $q < 0.05$. For brain volumes that showed significant effects of hormones, we then investigated the fixed effects of cycle phase in another mixed model where timepoint was an independent regressor of subfield volumes. We performed post hoc tests using the 'difflsmeans' function and the Satterthwaite correction for degrees of freedom. Participants were included as a random factor in all models. The R code for analyses is publicly available at https://github.com/RGZsido/MTLPlasticity2022.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this Article.

Data availability

Study preregistration is publicly available at https://osf.io/8mk74/. ASHS documentation, atlases and software, as well as the IKND Magdeburg Young Adult 7T Atlas, are available at https://sites.google.com/view/ashs-dox/ and https://www.nitrc.org/projects/ashs. Data are publicly available on an open data repository (https://doi.org/10.17617/3.2CPISW).

Code availability

All code for analyses is publicly available at https://github.com/RGZsido/MTLPlasticity2022.

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Author contributions

The principal investigator was J.S. The study was conceived and designed by R.G.Z., C.B., A.V. and J.S. Data acquisition was carried out by R.G.Z. and C.B. Data analysis was performed by R.G.Z., A.N.W., B.S. and L.K. Data were interpreted by R.G.Z., A.N.W., C.B., F.B., A.V.W., A.V. and J.S. T.M. and R.T. contributed to data analysis. The figures were created by R.G.Z. The original draft was written by R.G.Z. The manuscript was reviewed and edited by R.G.Z., A.N.W., C.B., B.S., L.K., T.M., R.T., F.B., A.V.W., A.V. and J.S.

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Competing interests

The authors declare no competing interests.

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Data analysis	-Automated Hippocampus SegmentationAlgorithm (ASHS), using the IKND Magdeburg Young Adult 7T Atlas, available: https://sites.google.com/view/ashs-dox/ and https://www.nitrc.org/projects/ashs - Segment Data module in CAT12 toolbox of SPM12 MATLAB R2021a - WFU PickAtlas toolbox - coregister:reslice function in SPM12 - Matlab Version R2021a - FLIRT and ANTS rigid registration algorithms - 'lmer' function in the 'lme4' R package (v3.5.2) - ITK-SNAP (v3.8) for quality assurance -Seaborn Python data visualization library based on matplotlib

All code for analyses is publicly available at https://github.com/RGZsido/MTLPlasticity2022 and https://github.com/JosePMarques/MP2RAGE-related-scripts. . ASHS documentation, atlases, and software are available at https://sites.google.com/view/ashs-dox/ and https://www.nitrc.org/projects/ashs. Data will be made publicly available on an open data repository (https://doi.org/10.17617/3.2CPISW) at the time of publication.

Participant sex hormone data analysis: "Estradiol and progesterone concentrations were determined using high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), and follicle-stimulating hormone and luteinizing hormone concentrations were determined using electrochemiluminescence immunoassay (ECLIA; Roche)."
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Research involving human participants, their data, or biological material

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Reporting on sex and gender	Our manuscript consistently uses the terms "sex" and "Female" to refer to our study population, as our central focus is on sex hormones as a biological variable and how fluctuations in endogenous estradiol/progesterone influence brain structure. Moreover, our inclusion criteria better capture the variable "sex" over "gender" (e.g., we do not include social and cultural variables, we do not provide the opportunity for self-identification on a spectrum) and our central focus is on sex hormones as a biological variable. We specifically chose to recruit an all female population given that sex hormones and female-specific variables, such as the menstrual cycle, influence the brain and the risk for mental health disorders. And while female individuals are at higher risk for cognitive and affective disorders (especially when ovarian hormones rapidly fluctuate and decline), there is a significant underrepresentation of female samples in neuroscience research, creating a huge data gap that limits opportunity for basic scientific discovery. Thus, we now provide the first longitudinal high-field MRI dataset of the hippocampus across the menstrual cycle, demonstrating the natural variance of female brain structural plasticity in a region instrumental to memory and affect regulation. This detailed neural phenotyping of brain areas substantially implicated in cognitive and neurodegenerative disease also allows for establishing an important reference: an integrative benchmark for evaluating female MRI biomarkers in health and disease and a prerequisite to ultimately assess depression and dementia risk later in life, pathologies which affect females twice as often as males and more frequently during times of a changing ovarian hormone environment
Reporting on race, ethnicity, or other socially relevant groupings	We reported in our manuscript about the ethnicity of our participants - this term does not represent any other variables. The definition of ethnicity derives from the participant. The information on ethnicity was self reported. In Germany, due to history and related ethical reasons, researchers do not ask questions on ethnicity to study participants. While this is unfortunate as this results in missing information on ethnicity, it was also the case in our study. All 27 female subjects were German without self-reported migration background or foreigner status. We thus conclude that the sample is likely of European/White ethnicity only. In Leipzig, 92 % of the population are Germans with a majority of European ancestry (estimated < 1.5% non-European ancestry such as Central/South-African or Asian ancestry). Of the 8 % non-German population, 4 % are male. Of the 4 % female Non-German population, only an estimated 1,5 % are within their reproductive years. While we made a designated effort (e.g., by specific recruitment strategies, e.g., reaching out with this request to local OB/GYNs to increase diversity of our study sample), we failed to find an eligible subject of probable Non European/Non-White ethnicity. We now include this information in the demographics table, as such.
Population characteristics	All participants were of reproductive age (mean SD, 25.33+3.64 years) with a healthy body mass index (BMI, 22.37‡2.69 kg/m2) and regular menstrual cycle length (29.04+2.62 days) (baseline demographics all reported in Table 1). Endogenous ovarian hormone values, subregion volumes, and whole hippocampus volume (sum of CA1, CA2, CA3, subiculum, dentate gyrus, and remaining tail) were within expected ranges (provided in Fig. 1, Table 2).
Recruitment	Figure 1 provides a flow diagram of screening, enrollment, assessment, and analysis. Participants from the general population were recruited with the use of advertisements and from our database of healthy volunteers. Following an initial telephone screening, volunteers set up an appointment time for a screening interview that includes clinical, laboratory and toxicology testing. One potential bias of the telephone screening may be that the quality of data is lower. On the other hand participants may be more open to reveal sensitive information. Eligible individuals were female, right-handed, 18-35 years old, with a BMI 18.5-29 kg/m2, and without any neurological or psychiatric illness as confirmed with a structured clinical interview. Exclusion criteria were prescription medication or supplement use, tobacco use, positive drug or pregnancy tests,

current use or use of hormonal contraceptives within the past 6 months, current or past hormone therapy, or having been pregnant, postpartum, breastfeeding, or had an abortion within one year of the study. Participants were screened and excluded for DSM-IV Axis I Disorders and Axis I Disorders, as well as for presence of premenstrual mood symptoms using the Premenstrual Symptoms Screening Tool (PSST).
All participants provided written informed consent after all procedures were fully explained. Participants were financially compensated for their participation.
Secondly, selection bias could emerge if participation is limited by hesitation to participate in multiple neuroimaging sessions and/or rigorous menstrual cycle monitoring procedures. Given the longitudinal design of the study and the multiple assessments, this cannot be ruled out. Strengths of this within-subject design include higher statistical power and better control of participants characteristics or individual differences, such as head motion affecting the individual study outcomes. We also acknowledge the possibility of attrition bias, which is inherent to protocols with multiple follow-up appointments. By careful randomization and controlling for socio-demographic and life-style factors, such as education-level and perceived stress, we aimed to limit the impact of such bias. Due to the majority of the population in Saxony being of German ethnicity, our sample is 100 percent European/German. As results from one population may not extrapolate well to another, we need further research to expand neuroimaging studies to enhance representation of multiple populations, and prioritize samples of Non-European descent. Finally, we studied an all female sample. This limits the generalizability of our findings to the male population, but provides relevant information that has been lacking in the neuroscience literature.

Ethics oversight

The Ethical Committee at the Medical Faculty of Leipzig University approved the study, protocol, and informed consent forms (#077-11-07032011).

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Life sciences study design

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Sample size

Assuming a medium effect size (n2 = 0.06), alpha coefficient of 0.05 and a power of 80 percent, we calculated a total sample size of N = 18 (a priori power analysis, G*Power). Based on the power calculations as well as previous literature in the field, we stated that we aimed to include N = 20 healthy participants with all six timepoints in the project protocol. We continued acquiring data until we had 20 participants with all 6 timepoints (of the included participants, twenty completed all six timepoints, two completed five timepoints, one completed three timepoints, one completed two timepoints, and three completed one timepoint. Dropout reasons listed below.).

Data exclusions

Figure 3 provides a flow diagram of screening, enrollment, assessment, and analysis. Forty-one participants were enrolled, of whom two were excluded due to inability to tolerate the 7T MRI scan, eight voluntarily discontinued due to time demands of study, and four were excluded due to irregular cycles, irregularities in bloodwork, or emergency contraceptive pill use after enrollment (included participants N = 27). Of the included participants, twenty completed all six timepoints, two completed five timepoints, one completed three timepoints, one completed two timepoints, and three completed one timepoint (dropout reasons: five due to scheduling conflicts, one used the emergency contraceptive pill, one got an MRI-incompatible retainer); for a total of 138 assessments.

Replication

The current study is a replication of our pilot experiment (n=1; Barth et al., 2016, Scientific Reports). Our 2016 manuscript shaped the hypothesis of this current manuscript ("Based on our pilot study (23), we hypothesized that cycle-related increases in estradiol levels would be associated with increases in whole hippocampus volume."). We essentially expanded the original study question (does hippocampus structure fluctuate with endogenous sex hormones) to include a larger sample, higher resolution MR imaging at ultra-high field strength, and more specific delineation of the hippocampus (e.g., segmentation of medial temporal lobe and hippocampus into subregions).

Randomization

The first assessment phase was randomized across participants, and all other assessments took place in remaining chronological order (menstrual, pre-ovulatory, ovulation, post-ovulatory, mid-luteal, premenstrual).

Blinding

N/A (no group allocation in this study). We do note that, during the statistical analysis phase, quality assurance images were visually assessed by two raters blinded to cycle phase.

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Outcomes	Blood: Serum from fasting-blood samples was collected at every ultrasound and assessment day visit to measure hormones, confirm cycle phase, ensure physical health, and exclude pregnancy or recent drug-intake. MRI: Anatomical MRI scans were acquired at the Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, using a Siemens Magnetom 7T system (Siemens Healthineers, Erlangen, Germany) and 32-channel head array coil (NOVA Medical Inc., Wilmington MA, USA), matched for time of day and without caffeine intake.

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☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	No task or resting-state MRI acquired; structural MR data described below.
Design specifications	No task or resting-state MRI acquired; structural MR data described below.
Behavioral performance measures	No task or resting-state MRI acquired; structural MR data described below.

Acquisition

Imaging type(s)	Structural MR data from 7T: whole-brain T1-weighted MP2RAGE sequence; T2-weighted TSE sequence. Cerebral blood flow MR data from 3T: T1-weighted MPRAGE sequence, pASL sequence.
Field strength	7T (for MP2RAGE and TSE, used for segmentation and volumetric analyses) and 3T (MPRAGE and pASL, used for cerebral blood flow calculations only)
Sequence & imaging parameters	MP2RAGE: TR=5000ms; TI 1/2=900/2750ms; TE=2.45ms; image matrix: 320×320×240; voxel size 0.7mm×0.7mmx0.7mm; flip angle 1/2=5°3°; parallel imaging using GRAPPA with acceleration factor=2) TSE: TR=16000m; TE=14ms; image matrix: 384×384; 50 slices; voxel size: 0.5mmx0.5mm×1mm; refocusing flip angle=120°; turbo factor=8; parallel imaging using GRAPPA with acceleration factor=2) MPRAGE: TR = 2300ms, TI = 900ms, TE = 4.21ms, flip angle =9°, FOV = 256 × 256mm, slices = 176, bandwidth =240Hz/px, voxel size = 1 mm 1 mmx 1mm PASL: TR = 3000ms, TII = 700ms, T1S = 1775, T12 = 1800ms, TE = 13ms, flip angle = 90°, matrix size = 64 x 64, slices = 24, FOV = 192mm x 192mm, voxel size = 3mm × 3mm x 4mm; labeling slab thickness = 100mm with a gap of 22mm, 101 pairs of label and control images
Area of acquisition	MP2RAGE, MPRAGE, and pAL were whole-brain. The T2-weighted TSE imaging slabs were acquired perpendicular to the anterior-posterior axis of the hippocampus. Segmentation of the medial temporal lobe into subregions was done using ASHS software and the Magdeburg Young Adult 7T Atlas.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	For main analyses: Segmentation and volume calculations for medial temporal lobe subregions were completed using ASHS. ASHS segmentat software uses a fully automated framework at all stages (MRI pre-processing, rigid body transformation alignment of T1- and T2-weighted images, bias correction and refining, etc.), automatically segmenting the medial temporal lobe in the T2-weighted MRI scans. ASHS documentation, atlases, and software are available at https://sites.google.com/

view/ashs-dox/ and https://www.nitrc.org/projects/ashs, with technical details and reliability described further in (Yushkevich et al., 2015, Human Brain Mapping).

Normalization: Please see above (ASHS segmentation software uses a fully automated framework at all stages)

Normalization template: Please see above (ASHS segmentation software uses a fully automated framework at all stages)

Noise and artifact removal: Please see above (ASHS segmentation software uses a fully automated framework at all stages). We also note that the chosen atlas (the Magdeburg Young Adult 7T Atlas) does exclude the alveus, fimbria, cerebrospinal fluid (CSF), and blood vessels. All quality assurance output images from ASHS were visually assessed by two raters blinded to cycle phase. We also performed extensive data quality checks: "Image quality was assessed using the overall image quality (IQ) (> 91%) as well as noise (> 91%), bias (> 91%), and image resolution (> 91%) parameters. Motion in the T1-weighted images was additionally assessed by detecting ghosting associated with head motion, in which the ghosting ratio should not exceed 3. Our values (mean SD, 1.1 +/-0.6) suggest that overall motion was negligible."

Volume censoring: For outlier detection in brain volumes, we flagged bilateral volumes that were three standard deviations from the mean for anatomical inspection by two raters. If upon inspection the volume segmentation maps were unanimously deemed anatomically sound, the volumes were kept to capture reasonable anatomical variation. Of the 138 assessments, we flagged a total of four brain volumes, of which two were determined to be of poor segmentation quality (both for CA1), and were thus removed from further analyses.

Statistical modeling & inference

Model type and settings: For control analyses, we performed linear mixed-effects modeling using the maximum likelihood method of the lmer function in the 'lme4' R package (V3.5.2) to assess potential effects of hormones (estradiol and progesterone) on CSF and cerebral blood flow. For main analyses, we used linear mixed-effects models to assess fixed effects of hormones as well as their interaction on' whole hippocampus and on each subregion volume. Inclusion of the interaction term was assessed by comparing model fits using the 'anova' function. The p-values of the model parameters were calculated via Wald tests and corrected for multiple comparisons using Benjamini-Hochberg procedure (80) controlling for FDR, and were accepted at an FDR-corrected threshold of $g < 0.05$. For brain volumes that showed significant effects of hormones, we then investigated the fixed effects of cycle phase timepoint modelled as an independent regressor, and performed post-hoc tests using the 'difflsmeans' fúnction and the Satterthwaite correction for degrees of freedom. Participants were included as a random factor in all models. The R code for analyses is publicly available at https://github.com/RGZsido/MTLPlasticity2022.

Effect(s) tested: ASHS and the Magdeburg Young Adult 7T Atlas automatically label and segment all subregions in the
locations)
medial temporal lobe. Whole hippocampus volume was calculated as the sum of CA1, CA2, CA3, subiculum, dentate gyrus, and remaining tail.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016): *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction: Corrected for multiple comparisons using Benjamini-Hochberg procedure controlling for FDR, and were accepted at an FDR-corrected threshold of $q < 0.05$.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity: *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis: *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis: *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*