Longitudinal 7T MRI reveals volumetric changes in subregions of human medial temporal lobe to sex hormone fluctuations

Rachel G. Zsido[1], Angharad N. Williams[1,2], Claudia Barth[3,4], Bianca Serio[1,5], Luisa Kurth[1], Frauke Beyer[1,6], A. Veronica Witte[1,6], Arno Villringer[1,5,6], Julia Sacher[1,5,6]

[1]Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany
[2]Department of Psychology, School of Social Sciences, Nottingham Trent University, Nottingham, United Kingdom
[3]Department of Psychiatric Research, Diakonhjemmet Hospital, Oslo, Norway
[4]Norwegian Centre for Mental Disorders Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[5]Max Planck School of Cognition, Leipzig, Germany
[6]Clinic for Cognitive Neurology, University Medical Center Leipzig, Germany

The hippocampus and surrounding medial temporal lobe (MTL) are critical for memory processes, with local atrophy linked to memory deficits. Animal work shows that MTL subregions densely express sex hormone receptors and exhibit rapid structural changes synchronized with hormone fluctuations. Such transient effects in humans have thus far not been shown. By combining a dense-sampling protocol, ultra-high field neuroimaging and individually-derived segmentation analysis, we demonstrate how estradiol and progesterone fluctuations affect MTL subregion volumes across the human menstrual cycle. Twenty-seven healthy women (19-34 years) underwent 7T MRI at six timepoints to acquire T1-weighted and T2-weighted images. Linear mixed-effects modeling showed positive associations between estradiol and parahippocampal cortex volume, progesterone and subiculum and perirhinal Area 35 volumes, and an estradiol*progesterone interaction with CA1 volume. We confirmed volumetric changes were not driven by hormone-related water (cerebral spinal fluid) or blood-flow (pulsed arterial spin labeling) changes. These findings suggest that sex hormones alter structural brain plasticity in subregions that are differentially sensitive to hormones. Mapping how endogenous endocrine factors shape adult brain structure has critical implications for women's health during the reproductive years as well as later in life, such as increased dementia risk following perimenopause, a period of pronounced sex hormone fluctuations.

Introduction

Ovarian hormones are powerful modulators of neuroplasticity, with animal research offering robust evidence of endocrine regulation of brain morphology on a rapid timescale (1, 2). 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 (2), dendritic spine and synapse density (3-6), mitochondrial and synaptic health (7, 8), and myelination (9, 10), 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 8-fold and progesterone levels 80-fold over a period of approximately 25-32 days (11). While a growing number of menstrual cycle studies suggest that ovarian hormone fluctuations do influence brain function and behavior in humans (12-15), 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 (16-18) and to be implicated in emotional regulation and cognition (2, 19-21), domains that are susceptible to cycle-dependent fluctuations (22, 23). The hippocampus and extended medial temporal lobe (MTL) are also rich in estradiol and progesterone receptors (24-26), and previous studies suggest that estradiol-dominant menstrual cycle phases are associated with greater hippocampal volume (27-29). Findings have been inconsistent, however, as menstrual cycle studies typically only assess two timepoints and do not directly measure ovarian hormone levels, rather using cycle phase as a proxy for hormone states (28, 29). In a single-subject pilot study (30), we observed that subtle gray matter density changes in the hippocampus paralleled daily fluctuations in endogenous estradiol levels across the menstrual cycle, a dynamic pattern that would have been overlooked with a sparse sampling approach. 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, while most human magnetic reasonance imaging (MRI) studies treat the hippocampus as a homogenous structure, recent advances in neuroimaging allow

for more precise delineation of neuroanatomical subregions of the hippocampus and MTL *in vivo* in humans (31-34). This specificity is critical given the unique cytoarchitecture, chemoarchitecture, and circuity of MTL subregions (34-36) that differentially contribute to aging and disease (37, 38). Subregion-specific architecture and circuitry, alongside potential differences in hormone receptor densities (39), suggest that hormone-modulated volumetric changes may manifest differently across the MTL complex. 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 (40) and in which neuronal loss has been associated with Alzheimer's disease (41). Estradiol enhances synaptogenesis and spine density in rodent and non-human primate CA1 neurons, while progesterone inhibits this effect (3, 5, 42, 43). Another study in female primates found that estradiol treatment increases pre- and post-synaptic proteins in CA1, while combined estradiol and progesterone decreases these synaptic proteins (44). Another region of key interest is perirhinal Area 35, corresponding to the transentorhinal region and medial perirhinal cortex (31, 45), in which atrophy has been associated with cognitive decline as well as early stages of dementia (45-49). While this subregion has received little attention in regards to endogenous hormone fluctuations, women have greater risk of developing Alzheimer's disease relative to men, particularly following periods of more pronounced hormone fluctuations later in life, such as after perimenopause (50-52). Thus, understanding how subtle hormone fluctuations may influence CA1 and Area 35 volume in young women would potentially provide insight into underlying mechanisms of risk for cognitive decline in women.

Initial evidence for hormone-modulated changes across human MTL subregions has been observed in a recent single-subject study using 3T MRI, in which the authors observed associations between daily ovarian hormone levels and MTL complex volumes (53). No study has yet to apply 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 brain, we provide a densly-sampled and detailed ultra-high field neuroimaging dataset in 27 healthy participants, who underwent 7T MRI scanning during six menstrual cycle phases: menstrual, pre-ovulatory, ovulation, post-ovulatory, mid-luteal, and premenstrual. We utilized

Automated Segmentation of Hippocampal Subfields software (ASHS (32)), which allows for a sensitive approach to individual differences in MTL subregion morphology (54). Notably, the chosen Magdeburg Young Adult 7T Atlas (31) leverages new information on anatomical variability, resulting in more sophisticated delineation of the boundary between the CA1 and subiculum as well as the parahippocampal cortex, and further segmentation of the perirhinal region into Areas 35 and 36. We developed a systematic protocol for rigorous cycle phase characterization to overcome inaccuracy of menstrual cycle monitoring, a limitation of previous work in this field (55, 56). Based on our pilot study (30), 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. The 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 as well as inform more individualized strategies for neuroimaging the MTL complex.

Results

Monitoring

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/m^2) and regular menstrual cycle length (29.04±2.62 days). Endogenous hormone values, subregion volumes, and whole hippocampus volume (sum of CA1, CA2, CA3, subiculum, dentate gyrus, and remaining tail) were within expected ranges (**Figure 1, Table 1**). 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).

Control analyses

Previous work in the field has been critiqued for not taking into account potential hormone-related water shifts or blood flow changes in the brain, which could be misconstrued as hormone-related brain volume changes. While the Magdeburg Young Adult 7T Atlas does exclude the alveus, fimbria, cerebrospinal fluid (CSF), and blood vessels, we additionally assessed CSF and blood flow changes in the hippocampus and did not observe statistically significant associations with estradiol (CSF: β = 4.28, 95% CI = -14 to 6, random effects SD = 22.62, p = 0.402) (blood flow: β = 1.23, 95% CI = -3 to 1, random effects SD = 4.19, p = 0.195) nor progesterone (CSF: β = 3.67, 95% CI = -1 to 8, random effects SD = 22.43, p = 0.134) (blood flow: β = 0.06, 95% CI = -1 to 1, random effects SD = 4.22, p = 0.899), giving further confidence that the following results were not erroneously driven by these factors.

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 (β = 108.26, 95% CI = 27 to 190, random effects SD = 174.47, p = 0.009). In MTL subregions, the addition of the estradiol*progesterone interaction to the model significantly improved model fit only for the CA1 ($\chi^2(1)$ = 7.691, p = 0.006) (**Figure 2A**). Estradiol was positively associated with CA1 volume (β = 42.87, 95% CI = 21 to 65, p < 0.001), progesterone was negatively associated with CA1 volume (β = -150.02, 95% CI = -249 to -51, p = 0.003), and we observed a significant interaction of estradiol and progesterone with CA1 volume (β = 53.06, 95% CI = 16 to 90, random effects SD = 44.03, p = 0.005), such that at higher progesterone levels, the positive effect of estradiol on CA1 volume is attenuated. Progesterone was positively associated with subiculum volume (β = 13.12, 95% CI = 4 to 22, random effects SD = 43.29, p = 0.006) (**Figure 2B**) and with Area 35 volume (β = 11.98, 95% CI = 2 to 21, random effects SD = 44.01, p = 0.014) (**Figure 2C**). Finally, estradiol was positively associated with parahippocampal cortex volume (β = 24.33, 95% CI = 10 to 39, random effects SD = 32.48, p = 0.001) (**Figure 2D**). All four subregions showed significant changes in volume over the six cycle phase timepoints (**Figure 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 ($0.196 \leq p$'s ≤ 0.884).

Discussion

In this study, we combined dense hormone sampling with individually-derived high resolution MTL segmentation analysis to demonstrate how estradiol and progesterone fluctuations affect 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 assess the hippocampus as a single homogenous structure. Finally, we provided further confidence that the observed effects do not erroneously stem from extra-neuronal factors, such as hormone-induced changes in regional blood flow or CSF expansion, as we did not find associations between these factors CSF and ovarian hormone levels. Taken together, our results suggest that ovarian hormones rapidly alter structural brain plasticity in subfields that 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 (41) and Area 35 (45-49), as women are more likely to suffer from cognitive impairment when ovarian hormones rapidly fluctuate, such as during perimenopause (50-52). 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 (3, 5, 8, 42-44). Moreover, progesterone administration decreases dendritic spines in rodent CA1 neurons, an effect that can be inhibited with a progesterone receptor antagonist (3). 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 (40). Clinical studies have also shown that estrogen replacement is associated with maintaining cognitive function in older age while progesterone may counteract the benefit of estradiol's cognitive enhancing effect (57-63). Thus, our 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.

We also observed a positive association between progesterone levels and Area 35 volume, which corresponds to the transentorhinal region and medial perirhinal cortex (31, 45). This region is clinically relevant with regards 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 (45-49). Given the increasing focus on the interplay between endogenous hormone fluctuations, the MTL, and the disproportionate risk for Alzheimer's disease in women (51, 52), we hypothesized that estradiol fluctuations would be associated with Area 35 volumetric changes. The observed progesterone association is a novel finding, as 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. Additional emphasis on progesterone in future research is warranted given that levels change approximately 80-fold over the menstrual cycle (11), and that both our current findings and previous work (53) show a complex role of progesterone on MTL subregion volumes. We note that, while the single-subject study (53) also observed both positive and negative associations between progesterone and subregion volumes, our observed progesterone findings occurred in different areas of the MTL, where the other study observed associations with CA2/3, perirhinal, entorhinal, and parahippocampal cortex volumes. These differences may be partially driven by differences in study design such as number of participants, scanner strength, segmentation atlas used, and differences in timepoints, and 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. While 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 (64) and after surgical menopause (65), respectively, 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 (37) and plays an important role in mediating hippocampal-cortical interactions (66), which are critically involved in cognitive function and emotional regulation, processes influenced by

menstrual cycle phase. While we highlight the relevant strengths of the methods used in this manuscript (e.g., atlas with distinction of Area 35, better boundary of subiculum and parahippocampal cortex (31)), 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 (2, 67), dendritic spine and synapse density (3-6), mitochondrial and synaptic health (7, 8), and myelination (9, 10), which can occur in a manner of hours-to-days. These hormone-induced actions can occur through activation of classical estrogen (ERα and ERβ) and progesterone receptors (PR), of which the MTL complex is dense with (24-26). For example, previous work has shown that ER antagonists and agonists can respectively decrease and increase cell proliferation in the dentate gyrus (68, 69). We note that we and others (44, 53) 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 prominent in the CA1 (39), where estradiol administration and ER agonists increase synaptic proteins (44, 70), ER antagonists decrease synaptic proteins (71), and progesterone administration and PR antagonists respectively decrease and increase dendritic spines (3, 5). These modulatory effects were not as prominent in the dentate gyrus and other subregions as compared to in CA1 (39, 44, 70, 71). Our study thus encourages future investigation of specific biological microstructural mechanisms underlying observed short-term dynamics of human MTL subregion morphology during times of ovarian hormone fluctuations.

While 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. Therefore, future studies should test whether these hormone-induced changes in MTL subregions are further exacerbated during times of more profound neuroendocrine change, and whether there are behavioral consequences for patient populations. 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 analysis (46-48, 72), and women are at increased risk for such disorders following ovarian hormone fluctuations, such as depressive disorders (73-77), multiple sclerosis (78-80), dementia (50, 52), and other inflammation-related disorders (81) during the premenstrual phase (73-75, 78, 81), postpartum period (73, 76, 79), and following perimenopause (50, 52, 73, 77, 80). Given the essential role of the MTL in adult synaptic plasticity and the differential contributions of MTL subregions to cognitive functions (40, 82-86), this information may be particularly relevant for plasticity-related disorders such as depression and dementia. Furthermore, 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 (35) 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 CA1 and subiculum, and that hippocampal projections to the medial prefrontal cortex (PFC) originate primarily in the CA1 and subiculum (87), 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 (88) and displays hormone-induced structural plasticity (4, 7, 8), with the hippocampal-prefrontal pathway implicated in cognitive and emotional processes (66, 87).

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 adult brain structural plasticity. This, alongside the current underrepresentation of female samples in neuroscience (55, 89-91), directly limits opportunities for basic scientific discovery and the diversity of human brain health. The MTL region has a remarkable degree of plasticity in response to subtle hormone changes across the female lifespan, and our findings suggest that these changes are detectable at the subregion level. To our knowledge, this is the first study to use ultra-high field neuroimaging to demonstrate

how endogenous hormone fluctuations rapidly and transiently alter volume across the MTL complex in multiple participants. We demonstrate the feasibility of such 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

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, use of hormonal contraceptives, 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 (92) and Axis II Disorders (93), as well as for presence of premenstrual mood symptoms using the Premenstrual Symptoms Screening Tool (PSST) (94). 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 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. The Ethical Committee at the Medical Faculty of Leipzig University approved the study, protocol, and informed consent forms (#077-11-07032011), and the study has been pre-registered at the Open Science Framework (https://osf.io/8mk74/).

Assessment timing

Participants had a documented history of regular menstrual cycles. The study assessments occurred during six cycle phases: menstrual (<5 days menses onset),

pre-ovulatory (≤2 days before ovulation), ovulation (≤24 hours of ovulation), post-ovulatory (≤2 days after ovulation), mid-luteal (6-8 days after ovulation), and premenstrual (≤3 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. To determine ovulatory timing, participants underwent multiple vaginal ultrasounds to track growing follicle and detect ovulation, completed luteinizing hormone urine tests throughout the ovulatory week, and consulted 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).

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 delivered immediately to the hospital laboratory and kept at 5°C until assayed within 24 hours. 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.

7T MRI acquisition

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. We acquired high-resolution whole-brain T1-weighted images using an MP2RAGE protocol (repetition time (TR) = 5000 ms; inversion time (TI) 1/2 = 900/2750 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 = 16000 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).

MTL segmentation and volumetry

Background noise removal from uniform T1-weighted MP2RAGE image volumes were done using https://github.com/JosePMarques/MP2RAGE-related-scripts (95). The high resolution T1- and T2-weighted images were then submitted to the Automatic Segmentation of Hippocampal Subfields (ASHS) package (32) using the Magdeburg Young Adult 7T Atlas (31), which has been shown to be more sensitive to individual differences in MTL subregion morphology compared to FreeSurfer (54). 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, etc.), automatically segmenting the MTL 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 (32). 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 Area 35 and Area 36]). ITK-SNAP (v3.8) was used for quality assurance (e.g., proper alignment of T1- and T2-weighted images). Quality assurance images were visually assessed by two raters blinded to cycle phase. All code is publicly available at https://github.com/RGZsido/MTLPlasticity2022.

Total brain volume, CSF, and cerebral blood flow

Total brain volume as well as CSF were calculated using the 7T T1-weighted images and the Segment Data module in CAT12 toolbox of SPM12 MATLAB R2021a, with all overall weighted image quality (IQR) measures > 90%. Cerebral blood flow was calculated using a T1-weighted MPRAGE (TR = 2300 ms, TI = 900 ms, TE = 4.21 ms, flip angle = 9°, FOV = 256 × 256 mm, slices = 176 , bandwidth = 240 Hz/px, voxel size = 1 × 1 × 1 mm^3) and a pulsed arterial spin labeling (pASL) sequence (TR = 3000,

TI1 = 700 ms, T1S = 1775, TI2 = 1800ms, TE = 13 ms, flip angle = 90°, matrix size = 64 × 64, slices = 24, FOV = 192 × 192 mm, voxel size = 3 × 3 × 4 mm^3; labeling slab thickness = 100 mm with a gap of 22 mm, 101 pairs of label and control images) (96), acquired on the same assessment days on a 3T Magnetom Verio scanner (Siemens, Erlangen, Germany) using a 32-channel head coil. The pASL data was preprocessed using an inhouse MATLAB analysis pipeline, which included co-registration to the MPRAGE image, motion correction with linear regression, normalization to MNI space, and smoothing with a 2D spatial Gaussian filter of 3-mm FWHM. The final cerebral blood flow values used for the analysis were calculated by pairwise subtraction of labeled and control images by the perfusion model (97).

For the cerebral blood flow analysis, an anatomical region-of-interest (ROI) was created as binary mask of the hippocampus using the WFU PickAtlas toolbox. The mask was resampled to a 3x3x4-mm voxel size to match the pASL images using the coregister:reslice function in SPM12. The preprocessed cerebral blood flow maps were multiplied with the binary mask of the hippocampus and the average cerebral blood flow 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), 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). 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 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. 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 (98)) 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 (99) 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 timepoint modelled as an independent regressor, and 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.

Acknowledgements

Preparation of this manuscript was supported by a Fellowship from the Joachim Herz Foundation (RGZ), the Branco Weiss Fellowship, Society in Science, National Association for Research on Schizophrenia and Depression (NARSAD) Young Investigator Grant 25032 from the Brain & Behavior Research Foundation (JS), and a Minerva Research Group Grant from the Max Planck Society (JS). The funders of the study had no role in study design, data collection, data interpretation, or writing of the report. The corresponding author had full access to all the data and final responsibility for the decision to submit for publication. We thank Matthias Heinrich for assistance in data acquisition, Toralf Mildner for assistance in pASL preprocessing, and Cornelia Ketscher and Heike Schmidt Duderstedt for assistance with data visualization.

Competing Interests

All authors declare that no competing interests exist.

References

1. Been LE, Sheppard PA, Galea LA, Glasper ER (2021): Hormones and neuroplasticity: A lifetime of adaptive responses. *Neuroscience & Biobehavioral Reviews*.
2. Barha CK, Galea LA (2010): Influence of different estrogens on neuroplasticity and cognition in the hippocampus. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 1800:1056-1067.
3. Woolley CS, McEwen BS (1993): Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol*. 336:293-306.
4. Hao J, Rapp PR, Leffler AE, Leffler SR, Janssen WG, Lou W, et al. (2006): Estrogen alters spine number and morphology in prefrontal cortex of aged female rhesus monkeys. *J Neurosci*. 26:2571-2578.
5. Woolley CS, McEwen BS (1992): Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat [published erratum appears in J Neurosci 1992 Oct; 12 (10): following table of contents]. *J Neurosci*. 12:2549-2554.
6. MacLusky NJ, Luine VN, Hajszan T, Leranth C (2005): The 17α and 17β isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariectomized female rats. *Endocrinology*. 146:287-293.
7. Hara Y, Yuk F, Puri R, Janssen WG, Rapp PR, Morrison JH (2014): Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. *Proceedings of the National Academy of Sciences*. 111:486-491.
8. Hara Y, Waters EM, McEwen BS, Morrison JH (2015): Estrogen effects on cognitive and synaptic health over the lifecourse. *Physiol Rev*. 95:785-807.
9. Patel R, Moore S, Crawford DK, Hannsun G, Sasidhar MV, Tan K, et al. (2013): Attenuation of corpus callosum axon myelination and remyelination in the absence of circulating sex hormones. *Brain Pathol*. 23:462-475.
10. Arevalo M-A, Santos-Galindo M, Bellini M-J, Azcoitia I, Garcia-Segura LM (2010): Actions of estrogens on glial cells: implications for neuroprotection. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 1800:1106-1112.
11. Stricker R, Eberhart R, Chevailler M-C, Quinn FA, Bischof P, Stricker R (2006): Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT® analyzer. *Clinical Chemistry and Laboratory Medicine (CCLM)*. 44:883-887.
12. Pritschet L, Santander T, Taylor CM, Layher E, Yu S, Miller MB, et al. (2020): Functional reorganization of brain networks across the human menstrual cycle. *Neuroimage*. 220:117091.
13. Weis S, Hodgetts S, Hausmann M (2019): Sex differences and menstrual cycle effects in cognitive and sensory resting state networks. *Brain Cogn*. 131:66-73.
14. Arélin K, Mueller K, Barth C, Rekkas PV, Kratzsch J, Burmann I, et al. (2015): Progesterone mediates brain functional connectivity changes during the menstrual cycle—a pilot resting state MRI study. *Frontiers in neuroscience*. 9:44.
15. Petersen N, Kilpatrick LA, Goharzad A, Cahill L (2014): Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. *Neuroimage*. 90:24-32.
16. Schmidt-Hieber C, Jonas P, Bischofberger J (2004): Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature*. 429:184-187.

513 17. Bartsch T, Wulff P (2015): The hippocampus in aging and disease: From plasticity to
514 vulnerability. *Neuroscience*. 309:1-16.
515 18. Sheppard PA, Choleris E, Galea LA (2019): Structural plasticity of the hippocampus in
516 response to estrogens in female rodents. *Molecular brain*. 12:1-17.
517 19. Frick KM, Kim J (2018): Mechanisms underlying the rapid effects of estradiol and
518 progesterone on hippocampal memory consolidation in female rodents. *Horm Behav*.
519 104:100-110.
520 20. Squire LR (1992): Memory and the hippocampus: a synthesis from findings with rats,
521 monkeys, and humans. *Psychol Rev*. 99:195.
522 21. Schumacher A, Villaruel FR, Ussling A, Riaz S, Lee AC, Ito R (2018): Ventral hippocampal
523 CA1 and CA3 differentially mediate learned approach-avoidance conflict processing. *Curr Biol*.
524 28:1318-1324. e1314.
525 22. Farage MA, Osborn TW, MacLean AB (2008): Cognitive, sensory, and emotional
526 changes associated with the menstrual cycle: a review. *Arch Gynecol Obstet*. 278:299-307.
527 23. Sundström Poromaa I, Gingnell M (2014): Menstrual cycle influence on cognitive
528 function and emotion processing—from a reproductive perspective. *Frontiers in neuroscience*.
529 8:380.
530 24. González M, Cabrera-Socorro A, Pérez-García CG, Fraser JD, López FJ, Alonso R, et al.
531 (2007): Distribution patterns of estrogen receptor α and β in the human cortex and
532 hippocampus during development and adulthood. *J Comp Neurol*. 503:790-802.
533 25. Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, et al. (2008):
534 Progesterone receptors: form and function in brain. *Front Neuroendocrinol*. 29:313-339.
535 26. Österlund MK, Grandien K, Keller E, Hurd YL (2000): The human brain has distinct
536 regional expression patterns of estrogen receptor α mRNA isoforms derived from alternative
537 promoters. *J Neurochem*. 75:1390-1397.
538 27. Lisofsky N, Mårtensson J, Eckert A, Lindenberger U, Gallinat J, Kühn S (2015):
539 Hippocampal volume and functional connectivity changes during the female menstrual cycle.
540 *Neuroimage*. 118:154-162.
541 28. Protopopescu X, Butler T, Pan H, Root J, Altemus M, Polanecsky M, et al. (2008):
542 Hippocampal structural changes across the menstrual cycle. *Hippocampus*. 18:985-988.
543 29. Pletzer B, Kronbichler M, Aichhorn M, Bergmann J, Ladurner G, Kerschbaum HH (2010):
544 Menstrual cycle and hormonal contraceptive use modulate human brain structure. *Brain Res*.
545 1348:55-62.
546 30. Barth C, Steele CJ, Mueller K, Rekkas VP, Arélin K, Pampel A, et al. (2016): In-vivo
547 dynamics of the human hippocampus across the menstrual cycle. *Scientific reports*. 6:1-9.
548 31. Berron D, Vieweg P, Hochkeppler A, Pluta J, Ding S-L, Maass A, et al. (2017): A protocol
549 for manual segmentation of medial temporal lobe subregions in 7 Tesla MRI. *NeuroImage:*
550 *Clinical*. 15:466-482.
551 32. Yushkevich PA, Pluta JB, Wang H, Xie L, Ding SL, Gertje EC, et al. (2015): Automated
552 volumetry and regional thickness analysis of hippocampal subfields and medial temporal
553 cortical structures in mild cognitive impairment. *Hum Brain Mapp*. 36:258-287.
554 33. Ding SL, Royall JJ, Sunkin SM, Ng L, Facer BA, Lesnar P, et al. (2016): Comprehensive
555 cellular-resolution atlas of the adult human brain. *J Comp Neurol*. 524:3127-3481.
556 34. Ding SL, Van Hoesen GW (2015): Organization and detailed parcellation of human
557 hippocampal head and body regions based on a combined analysis of cyto-and
558 chemoarchitecture. *J Comp Neurol*. 523:2233-2253.
559 35. Duvernoy HM, Cattin F, Risold P-Y (2005): *The human hippocampus: functional*
560 *anatomy, vascularization and serial sections with MRI*. Springer.

561 36. Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah N, et al. (2005):
562 Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal
563 cortex: intersubject variability and probability maps. *Anat Embryol (Berl)*. 210:343-352.
564 37. Malykhin NV, Huang Y, Hrybouski S, Olsen F (2017): Differential vulnerability of
565 hippocampal subfields and anteroposterior hippocampal subregions in healthy cognitive
566 aging. *Neurobiol Aging*. 59:121-134.
567 38. de Flores R, La Joie R, Landeau B, Perrotin A, Mézenge F, de La Sayette V, et al. (2015):
568 Effects of age and Alzheimer's disease on hippocampal subfields: comparison between manual
569 and FreeSurfer volumetry. *Hum Brain Mapp*. 36:463-474.
570 39. Mitterling KL, Spencer JL, Dziedzic N, Shenoy S, McCarthy K, Waters EM, et al. (2010):
571 Cellular and subcellular localization of estrogen and progestin receptor immunoreactivities in
572 the mouse hippocampus. *J Comp Neurol*. 518:2729-2743.
573 40. Schlichting ML, Zeithamova D, Preston AR (2014): CA1 subfield contributions to
574 memory integration and inference. *Hippocampus*. 24:1248-1260.
575 41. West MJ, Coleman PD, Flood DG, Troncoso JC (1994): Differences in the pattern of
576 hippocampal neuronal loss in normal ageing and Alzheimer's disease. *The Lancet*. 344:769-
577 772.
578 42. Hao J, Janssen WG, Tang Y, Roberts JA, McKay H, Lasley B, et al. (2003): Estrogen
579 increases the number of spinophilin-immunoreactive spines in the hippocampus of young and
580 aged female rhesus monkeys. *J Comp Neurol*. 465:540-550.
581 43. Bali N, Arimoto JM, Iwata N, Lin SW, Zhao L, Brinton RD, et al. (2012): Differential
582 responses of progesterone receptor membrane component-1 (Pgrmc1) and the classical
583 progesterone receptor (Pgr) to 17β-estradiol and progesterone in hippocampal subregions
584 that support synaptic remodeling and neurogenesis. *Endocrinology*. 153:759-769.
585 44. Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z, McEwen BS (2003): Estradiol
586 increases pre-and post-synaptic proteins in the CA1 region of the hippocampus in female
587 rhesus macaques (Macaca mulatta). *Endocrinology*. 144:4734-4738.
588 45. Braak H, Braak E (1991): Neuropathological stageing of Alzheimer-related changes.
589 *Acta Neuropathol (Berl)*. 82:239-259.
590 46. Olsen RK, Yeung L-K, Noly-Gandon A, D'Angelo MC, Kacollja A, Smith VM, et al. (2017):
591 Human anterolateral entorhinal cortex volumes are associated with cognitive decline in aging
592 prior to clinical diagnosis. *Neurobiol Aging*. 57:195-205.
593 47. Berron D, Vogel JW, Insel PS, Pereira JB, Xie L, Wisse LE, et al. (2021): Early stages of
594 tau pathology and its associations with functional connectivity, atrophy and memory. *Brain*.
595 144:2771-2783.
596 48. Krumm S, Kivisaari SL, Probst A, Monsch AU, Reinhardt J, Ulmer S, et al. (2016): Cortical
597 thinning of parahippocampal subregions in very early Alzheimer's disease. *Neurobiol Aging*.
598 38:188-196.
599 49. Ding SL, Van Hoesen GW, Cassell MD, Poremba A (2009): Parcellation of human
600 temporal polar cortex: a combined analysis of multiple cytoarchitectonic, chemoarchitectonic,
601 and pathological markers. *J Comp Neurol*. 514:595-623.
602 50. Brinton RD, Yao J, Yin F, Mack WJ, Cadenas E (2015): Perimenopause as a neurological
603 transition state. *Nature reviews endocrinology*. 11:393-405.
604 51. Georgakis MK, Beskou-Kontou T, Theodoridis I, Skalkidou A, Petridou ET (2019):
605 Surgical menopause in association with cognitive function and risk of dementia: a systematic
606 review and meta-analysis. *Psychoneuroendocrinology*. 106:9-19.

607 52. Lee BH, Puri TA, Galea LA (2021): Sex and sex hormone differences in hippocampal
608 neurogenesis and their relevance to Alzheimer's disease. *Sex and Gender Differences in*
609 *Alzheimer's Disease*: Elsevier, pp 23-77.
610 53. Taylor CM, Pritschet L, Olsen RK, Layher E, Santander T, Grafton ST, et al. (2020):
611 Progesterone shapes medial temporal lobe volume across the human menstrual cycle.
612 *Neuroimage*. 220:117125.
613 54. Sone D, Sato N, Maikusa N, Ota M, Sumida K, Yokoyama K, et al. (2016): Automated
614 subfield volumetric analysis of hippocampus in temporal lobe epilepsy using high-resolution
615 T2-weighed MR imaging. *NeuroImage: Clinical*. 12:57-64.
616 55. Taylor CM, Pritschet L, Jacobs EG (2021): The scientific body of knowledge–Whose
617 body does it serve? A spotlight on oral contraceptives and women's health factors in
618 neuroimaging. *Front Neuroendocrinol*. 60:100874.
619 56. Schmalenberger KM, Tauseef HA, Barone JC, Owens SA, Lieberman L, Jarczok MN, et
620 al. (2021): How to study the menstrual cycle: Practical tools and recommendations.
621 *Psychoneuroendocrinology*. 123:104895.
622 57. Rice MM, Graves AB, McCurry SM, Gibbons LE, Bowen JD, McCormick WC, et al. (2000):
623 Postmenopausal estrogen and estrogen-progestin use and 2-year rate of cognitive change in
624 a cohort of older Japanese American women: The Kame Project. *Arch Intern Med*. 160:1641-
625 1649.
626 58. Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, et al.
627 (2003): Effect of estrogen plus progestin on global cognitive function in postmenopausal
628 women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA*.
629 289:2663-2672.
630 59. Jacobs DM, Tang M-X, Stern Y, Sano M, Marder K, Bell K, et al. (1998): Cognitive
631 function in nondemented older women who took estrogen after menopause. *Neurology*.
632 50:368-373.
633 60. Yesufu A, Bandelow S, Hogervorst E (2007): Meta-analyses of the effect of hormone
634 treatment on cognitive function in postmenopausal women. *Women's Health*. 3:173-194.
635 61. Phillips SM, Sherwin BB (1992): Effects of estrogen on memory function in surgically
636 menopausal women. *Psychoneuroendocrinology*. 17:485-495.
637 62. Sherwin BB (1998): Estrogen and cognitive functioning in women. *Proc Soc Exp Biol*
638 *Med*. 217:17-22.
639 63. Sherwin BB (1997): Estrogen effects on cognition in menopausal women. *Neurology*.
640 48:21S-26S.
641 64. Luders E, Gaser C, Gingnell M, Engman J, Sundström Poromaa I, Kurth F (2021): Gray
642 matter increases within subregions of the hippocampal complex after pregnancy. *Brain*
643 *Imaging and Behavior*. 15:2790-2794.
644 65. Zeydan B, Tosakulwong N, Schwarz CG, Senjem ML, Gunter JL, Reid RI, et al. (2019):
645 Association of bilateral salpingo-oophorectomy before menopause onset with medial
646 temporal lobe neurodegeneration. *JAMA neurology*. 76:95-100.
647 66. Godsil BP, Kiss JP, Spedding M, Jay TM (2013): The hippocampal–prefrontal pathway:
648 the weak link in psychiatric disorders? *Eur Neuropsychopharmacol*. 23:1165-1181.
649 67. Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP (2000):
650 Antiinflammatory effects of estrogen on microglial activation. *Endocrinology*. 141:3646-3656.
651 68. Mazzucco C, Lieblich S, Bingham B, Williamson M, Viau V, Galea L (2006): Both estrogen
652 receptor α and estrogen receptor β agonists enhance cell proliferation in the dentate gyrus of
653 adult female rats. *Neuroscience*. 141:1793-1800.

69. Nagy AI, Ormerod BK, Mazzucco C, Galea LA (2005): Estradiol-induced enhancement in cell proliferation is mediated through estrogen receptors in the dentate gyrus of adult female rats. *Drug development research*. 66:142-149.
70. Waters EM, Mitterling K, Spencer JL, Mazid S, McEwen BS, Milner TA (2009): Estrogen receptor alpha and beta specific agonists regulate expression of synaptic proteins in rat hippocampus. *Brain Res*. 1290:1-11.
71. Brake WG, Alves SE, Dunlop JC, Lee SJ, Bulloch K, Allen PB, et al. (2001): Novel target sites for estrogen action in the dorsal hippocampus: an examination of synaptic proteins. *Endocrinology*. 142:1284-1289.
72. Wisse LE, Biessels GJ, Heringa SM, Kuijf HJ, Luijten PR, Geerlings MI, et al. (2014): Hippocampal subfield volumes at 7T in early Alzheimer's disease and normal aging. *Neurobiol Aging*. 35:2039-2045.
73. Deecher D, Andree TH, Sloan D, Schechter LE (2008): From menarche to menopause: exploring the underlying biology of depression in women experiencing hormonal changes. *Psychoneuroendocrinology*. 33:3-17.
74. Epperson CN, Steiner M, Hartlage SA, Eriksson E, Schmidt PJ, Jones I, et al. (2012): Premenstrual dysphoric disorder: evidence for a new category for DSM-5. *Am J Psychiatry*. 169:465-475.
75. Rapkin AJ, Akopians AL (2012): Pathophysiology of premenstrual syndrome and premenstrual dysphoric disorder. *Menopause international*. 18:52-59.
76. Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T (2005): Perinatal depression: a systematic review of prevalence and incidence. *Obstetrics & Gynecology*. 106:1071-1083.
77. Freeman EW, Sammel MD, Boorman DW, Zhang R (2014): Longitudinal pattern of depressive symptoms around natural menopause. *JAMA psychiatry*. 71:36-43.
78. ZORGDRAGER A, DE KEYSER J (1998): Premenstrual exacerbations of multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*. 65:279-280.
79. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T, Group PiMS (1998): Rate of pregnancy-related relapse in multiple sclerosis. *N Engl J Med*. 339:285-291.
80. Ramagopalan SV, Dobson R, Meier UC, Giovannoni G (2010): Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *The Lancet Neurology*. 9:727-739.
81. Alvergne A, Tabor VH (2018): Is female health cyclical? Evolutionary perspectives on menstruation. *Trends in ecology & evolution*. 33:399-414.
82. Travis S, Huang Y, Fujiwara E, Radomski A, Olsen F, Carter R, et al. (2014): High field structural MRI reveals specific episodic memory correlates in the subfields of the hippocampus. *Neuropsychologia*. 53:233-245.
83. Ogawa M, Sone D, Beheshti I, Maikusa N, Okita K, Takano H, et al. (2019): Association between subfield volumes of the medial temporal lobe and cognitive assessments. *Heliyon*. 5:e01828.
84. Lee AC, Buckley MJ, Pegman SJ, Spiers H, Scahill VL, Gaffan D, et al. (2005): Specialization in the medial temporal lobe for processing of objects and scenes. *Hippocampus*. 15:782-797.
85. Inhoff MC, Ranganath C (2015): Significance of objects in the perirhinal cortex. *Trends in Cognitive Sciences*. 19:302-303.
86. Aminoff EM, Kveraga K, Bar M (2013): The role of the parahippocampal cortex in cognition. *Trends in cognitive sciences*. 17:379-390.

87. Jin J, Maren S (2015): Prefrontal-hippocampal interactions in memory and emotion. *Frontiers in systems neuroscience*. 9:170.
88. Wang AC, Hara Y, Janssen WG, Rapp PR, Morrison JH (2010): Synaptic estrogen receptor-α levels in prefrontal cortex in female rhesus monkeys and their correlation with cognitive performance. *J Neurosci*. 30:12770-12776.
89. Beery AK, Zucker I (2011): Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews*. 35:565-572.
90. Will TR, Proaño SB, Thomas AM, Kunz LM, Thompson KC, Ginnari LA, et al. (2017): Problems and progress regarding sex bias and omission in neuroscience research. *eneuro*. 4.
91. Garcia-Sifuentes Y, Maney DL (2021): Reporting and misreporting of sex differences in the biological sciences. *ELife*. 10:e70817.
92. Wittchen H-U, Wunderlich U, Gruschwitz S, Zaudig M (1997): SKID I. Strukturiertes Klinisches Interview für DSM-IV. Achse I: Psychische Störungen. Interviewheft und Beurteilungsheft. Eine deutschsprachige, erweiterte Bearb. d. amerikanischen Originalversion des SKID I.
93. Fydrich T, Renneberg B, Schmitz B, Wittchen H-U (1997): SKID II. Strukturiertes Klinisches Interview für DSM-IV, Achse II: Persönlichkeitsstörungen. Interviewheft. Eine deutschspeachige, erw. Bearb. d. amerikanischen Originalversion d. SKID-II von: MB First, RL Spitzer, M. Gibbon, JBW Williams, L. Benjamin,(Version 3/96).
94. Steiner M, Macdougall M, Brown E (2003): The premenstrual symptoms screening tool (PSST) for clinicians. *Archives of Women's Mental Health*. 6:203-209.
95. O'Brien KR, Kober T, Hagmann P, Maeder P, Marques J, Lazeyras F, et al. (2014): Robust T1-weighted structural brain imaging and morphometry at 7T using MP2RAGE. *PLoS ONE*. 9:e99676.
96. Luh WM, Wong EC, Bandettini PA, Hyde JS (1999): QUIPSS II with thin-slice TI1 periodic saturation: a method for improving accuracy of quantitative perfusion imaging using pulsed arterial spin labeling. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine*. 41:1246-1254.
97. Wong EC, Buxton RB, Frank LR (1998): A theoretical and experimental comparison of continuous and pulsed arterial spin labeling techniques for quantitative perfusion imaging. *Magn Reson Med*. 40:348-355.
98. RCore T (2013): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing [Internet]. Vienna, Austria.
99. Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*. 57:289-300.

Figures

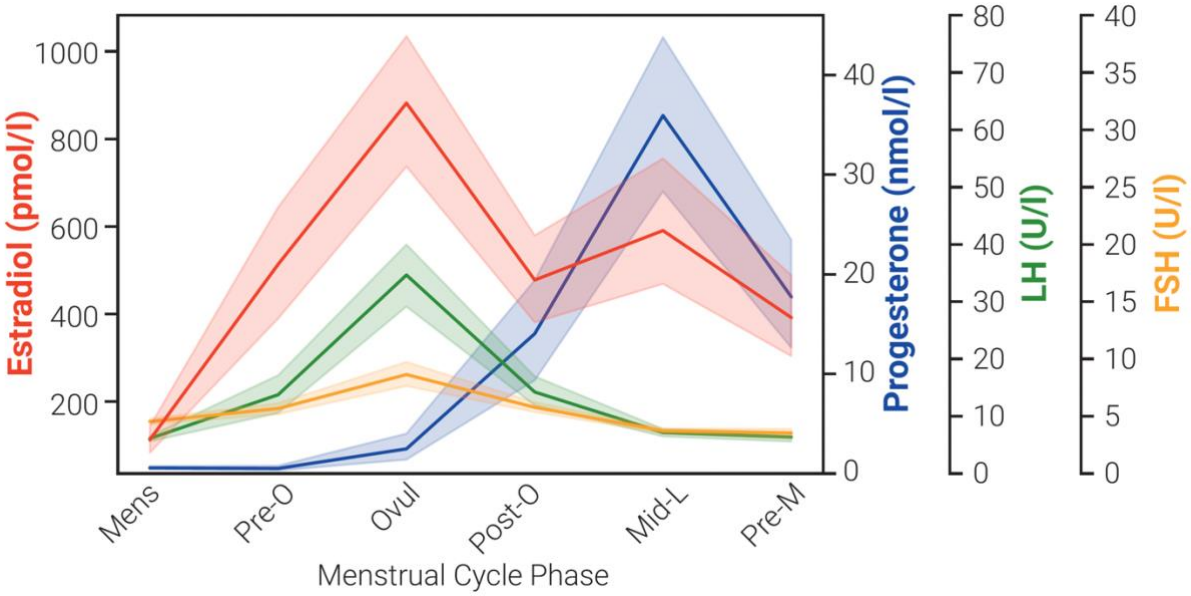

Figure 1: Changes in endogenous levels of estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) across menstrual cycle. Shaded area represents 95% confidence interval for hormone levels at menstrual (Mens), pre-ovulatory (Pre-O), ovulation (Ovul), post-ovulatory (Post-O), mid-luteal (Mid-L), and premenstrual (Pre-M) timepoints.

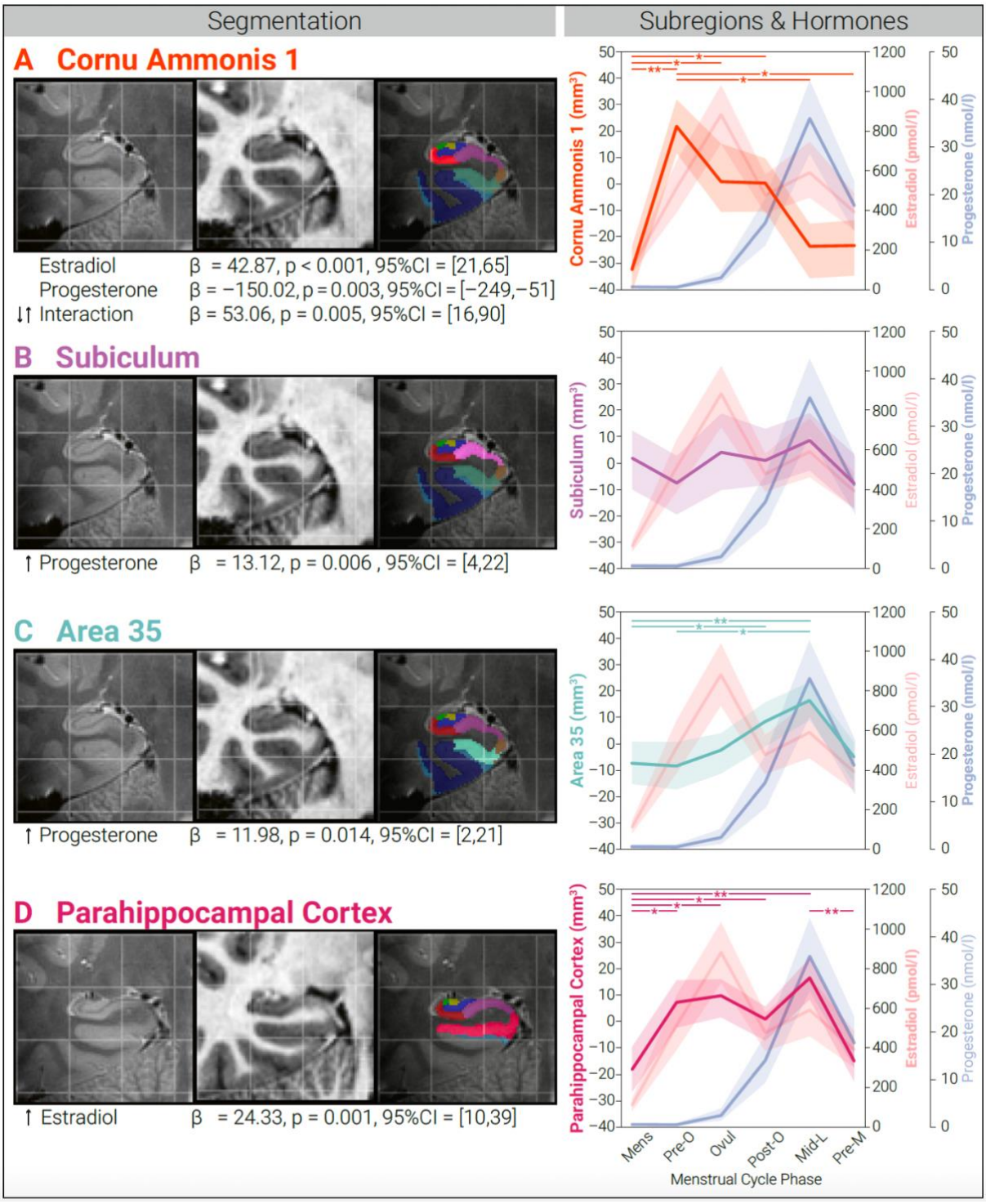

Figure 2: Changes in medial temporal lobe (MTL) volume associated with ovarian hormones across menstrual cycle. *Column 1*: Example T2-weighted image, T1-weighted image, and MTL segmentation for (**A**) cornu ammonis 1, (**B**) subiculum, (**C**) perirhinal Area 35, and (**D**) parahippocampal cortex. *Column 2*: After segmentation, unique associations between ovarian hormones and MTL regions across menstrual cycle. Linear mixed-effects model statistics reported in Column 2. Shaded area represents 95% confidence interval for hormone levels or subregion volumes. Asterisks refer to statistically significant changes in volume over timepoints, **p*<0.05, ***p*<0.005.

762 **Tables**

763

	Mean (mm^3)	SD (mm^3)	Range (mm^3)
Total Brain Volume	1130246.38	83996.77	412000.00
Whole Hippocampus	5295.36	381.71	2322.00
CA1	1399.73	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	2043.41	146.34	739.25
Entorhinal Cortex	1151.98	165.34	852.50
Area 35	752.80	104.35	585.25
Area 36	4046.27	543.87	1959.25
Parahippocampal Cortex	660.92	97.75	540.00

764

765 **Table 1 Descriptive statistics for each brain region volume, mm^3.** Whole
766 hippocampus is the sum of cornu ammonis [CA] 1, CA2, CA3, subiculum, dentate
767 gyrus, and remaining tail. Standard deviation [SD].