Variability in human hippocampal subfield volume across one complete reproductive cycle

C.M. Taylor[1], L. Pritschet[1], E. Layher[1], T. Santander[1], S.T. Grafton[1], E.G. Jacobs[1,2]

[1]Department of Psychological and Brain Sciences [2]Neuroscience Research Institute University of California, Santa Barbara

UCSB BRAIN INITIATIVE

INTRODUCTION

- The brain is an endocrine organ.
  - Estradiol (E) and progesterone (P) signaling are critical components of cell survival and plasticity[1].
  - E and P receptors are expressed throughout the brain, with high receptor density in the hippocampus, a region critical for higher-order cognitive function[2] .
- Women experience significant fluctuations in serum sex hormone levels across the menstrual cycle (12-fold in E, 800-fold in P).
- Despite this feature of ~50% of the world's population, we have limited understanding of the effects of the menstrual cycle on the brain (e.g. hippocampal morphology).
  - Higher levels of E correspond with larger hippocampal volumes in animal models[3,4,5,6].
  - In humans, studies suggest that hippocampal volume changes across the menstrual cycle[7,8].
  - Changes in sex hormone levels at menopause are associated with changes in hippocampal subfield volumes[9,10].
- While compelling, these findings are based on comparisons between only 2-3 time points across a 28 day cycle, or between groups of different hormonal status.
- Recent approaches in neuroscience have moved towards densely sampling individuals to understand sources of intra-subject variability in brain structure[11], e.g. fluctuations in brain volume across a menstrual cycle[12].
- In this deep-phenotyping study, **we examined the relationship between hippocampal subfield volumes and endogenous fluctuations in sex steroid hormones across 30 consecutive days in a healthy, naturally cycling female**.

METHODS

PARTICIPANT: The participant (author LP) is a right-handed Caucasian female, aged 23 years old at the onset of the study. She is a healthy, regularly and naturally cycling woman, with no history of neuropsychiatric or endocrine disorders.

DATA COLLECTION: LP underwent time-locked (±30 min) daily blood draws and MRI scans for 30 consecutive days. Venous blood sampling took place each morning to evaluate serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), 17β-estradiol (E), and progesterone (P) via liquid chromatography-mass spectrometry, conducted at the Brigham and Women's Research Assay Core.

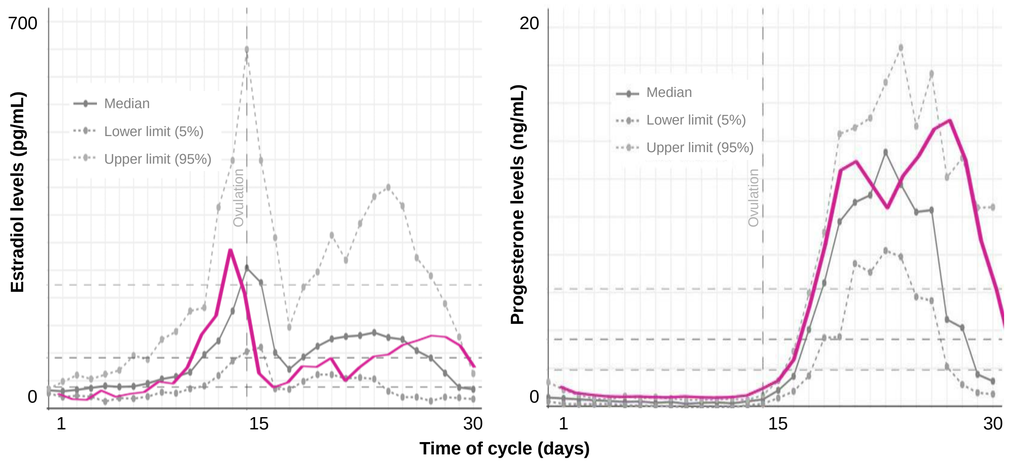

Figure 1. Participant's estradiol (left) and progesterone (right) concentrations (pink) plotted against median (solid gray line) and 5th/95th percentiles (dotted gray lines) hormone concentrations based on serum samples from 20 naturally cycling females[13].

HIPPOCAMPAL SEGMENTATION: MRIs were acquired on a Siemens 3T Prisma scanner at the UCSB Brain Imaging Center, including high resolution T2- (31 oblique slices, TR= 8100 ms, TE=50ms, 0.4 x 0.4 x 2 mm) and T1-weighted scans. The automatic segmentation of hippocampal subfields package (ASHS[14]) was used to segment the hippocampus and surrounding cortex into seven subfields (Fig 1). Each T2 image was aligned with the same day's T1 scan, which was then registered to a population template (the Princeton Young Adult 3T Atlas for New ASHS) prior to the segmentation and calculation of subfield volumes. In order to determine whether hippocampal volumes were related with fluctuations in sex serum hormone levels, we calculated Pearson's correlation coefficients between subfield volumes (left and right) and E or P. Given the bimodal distribution of P values, we also conducted two-sample *t*-tests between subfield volumes on cycle days 1-15 (low P) and cycle days 16-30 (high P). Correlations were considered significant if they met a Bonferroni-corrected threshold (0.05/14 subfields = $p < 0.0036$).

RESULTS

Automatic Segmentation of Hippocampal Subfields (ASHS)

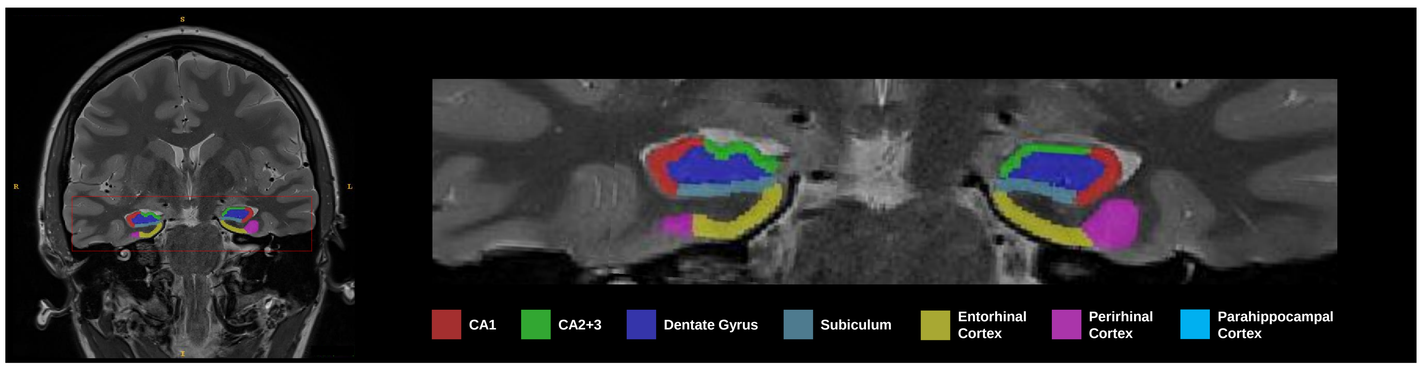

Figure 2. Sample slice depicting ASHS segmentation.

Hippocampal subfield volumes fluctuate across the menstrual cycle

Table 2. Summary of hippocampal subfield volumes across the 30 day study period.

	CA1	CA2+3	DG	Sub	ERC	PRC	PHC		CA1	CA2+3	DG	Sub	ERC	PRC	PHC
Left Hemisphere								**Right Hemisphere**							
Average (SD)	732.3 (19.5)	223.5 (16.7)	816.0 (26.0)	492.7 (26.7)	613.8 (36.1)	1876.4 (276.2.)	2360.9 (76.2)	**Average (SD)**	881.4 (19.2)	355.3 (20.8)	859.7 (16.6)	561.0 (25.1)	776.0 (44.8)	1379.5 (182.3)	2308.4 (40.2)
Min	696.7	179.7	769.3	430.3	529.8	1332.7	2199.1	**Min**	835.9	316.8	835.0	505.1	661.0	955.2	2215.6
Max	776.7	248.1	877.4	551.8	672.3	2323.0	2511.3	**Max**	920.4	411.7	901.2	609.4	836.5	1585.4	2389.0
Range (% of Max Volume)	80.0 (10.3)	68.4 (27.6)	108.0 (12.3)	121.5 (22.0)	142.5 (21.2)	990.3 (42.6)	312.2 (12.4)	**Range (% of Max Volume)**	84.5 (9.2)	94.9 (23.1)	66.2 (7.3)	104.4 (17.1)	175.5 (21.0)	630.2 (39.7)	173.4 (7.3)
Correlation with E *p*-value (*r*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**Correlation with E *p*-value (*r*)**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.026 (0.41)
Correlation with P *p*-value (*r*)	n.s.	n.s.	0.009 (0.47)	n.s.	0.0005* (-0.60)	0.00009* (-0.65)	0.006 (0.49)	**Correlation with P *p*-value (*r*)**	0.003 (-0.52)	0.048 (0.36)	n.s.	n.s.	0.0001* (-0.64)	0.0028* (-0.53)	n.s.

* indicates significance at Bonferroni corrected $p < 0.0036$

Entorhinal and Perirhinal cortex volume is related to Progesterone

Estradiol – Estradiol concentration positively correlated with right parahippocampal cortex volume, but this relationship did not survive Bonferroni correction ($p < 0.0036$).

Progesterone – Progesterone was positively correlated with volumes within left dentate gyrus and parahippocampal cortex as well as right CA2-3, and negatively correlated with right CA1 volume, but these did not survive Bonferroni correction ($p < 0.0036$).

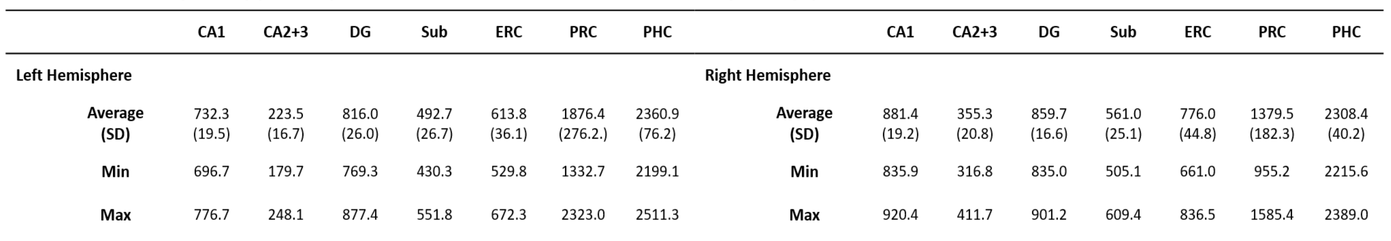

Figure 3a-d. Subfield volumes vs. Progesterone. P was significantly negatively correlated with GMV in bilateral entorhinal cortex (**a.** left: $r = -0.60$, $p < 0.001$; **b.** right: $r = -0.64$, $p < 0.001$), and in bilateral perirhinal cortex (**c.** left: $r = -0.65$, $p < 0.001$; **d.** right: $r = -0.53$, $p < 0.003$). These results remained significant after non-parametric spearman rank-coefficient correlations (all $p < 0.05$). As P was significantly correlated with global GMV ($p = 0.04$, $r = 0.38$), we conducted partial correlations to control for this relationship and all P vs. subfield relationships remained significant (all $p < 0.01$).

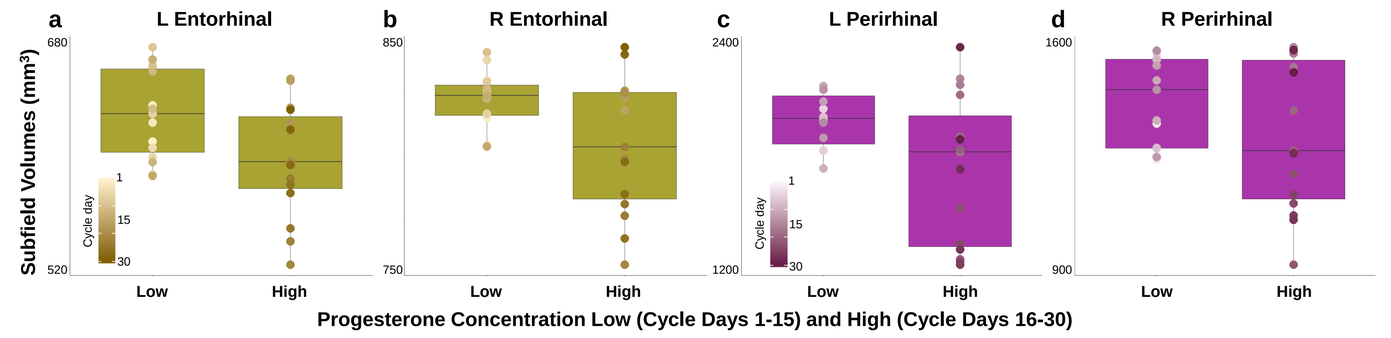

Figure 4a-d. Subfield volumes with Low Progesterone vs. High Progesterone. Bilateral entorhinal cortex **(a, b)** and bilateral perirhinal cortex volumes **(c, d)** on cycle days 1-15 (Low P) were significantly larger than those on cycle days 16-30 (High P).

CONCLUSIONS & DISCUSSION

CONCLUSIONS

- Serum concentrations of E and P were within expected ranges[13], and showed the canonical fluctuations across the menstrual cycle, with E peaking in late follicular phase and P concentrations rising dramatically during the mid-luteal phase.
- **Hippocampal volume (total and within subfields) changed across the 30-day study period**, with fluctuations ranging from 7-40% of maximum subfield volume.
- Estrogen had no significant relationship with hippocampal subfield volumes, with a trending positive relationship between E and R parahippocampal cortex volume.
- **Progesterone was significantly negatively correlated with bilateral entorhinal and perirhinal volumes**, with trending positive correlations with left dentate gyrus, parahippocampal, and right CA2-3 volumes, and negative correlations with right CA1 volume.

DISCUSSION

- These findings build on animal work demonstrating a dramatic inhibitory effect of progesterone on hippocampal morphology across the 5-day rat estrous cycle[15], with progesterone reversing the neurotrophic effects of estradiol in entorhinal cortex, specifically[16].
- The entorhinal and perirhinal cortices, considered a functional unit in medial temporal lobe circuitry[17], are the first sites of Alzheimer's (AD) pathophysiology[18], and there is higher incidence and prevalence of AD in women (reviewed in [19]).
- Our findings suggest that the relationship between sex hormones (progesterone) and these subfields is dynamic throughout reproductive life and should be probed.

REFERENCES

1. Galea *et al. Neurosci Biobehav Rev. 76*, 363-379 (2017). 2. Rossetti *et al. Journal of neuroendocrinol*, *28* (2016). 3. Woolley & McEwen. *J Neurosci.* 12, 2549-2554 (1992). 4. Hao *et al. J Comp Neurol*, *465*, 540-550 (2003). 5. Tang *et al. Cereb Cortex*, *14*, 215-223 (2004). 6. Qiu *et al. NeuroImage*, *83*, 593-598 (2013). 7. Lisofsky *et al. NeuroImage*, *118*, 154-162. (2015). 8. Protopopescu *et al. Hippocampus*, *18*, 985-988 (2008). 9. Gervais *et al.* (2018). *Alzheimers Dement*, *14*, P1234 (2018). 10. Zeydan *et al. JAMA Neurol*, *76*, 95-100 (2019). 11. Poldrack *et al. Nat Commun*, *6*, 8885 (2015). 12. Arélin *et al. Front Neurosci*, *9*, 44 (2015). 13. Stricker *et al. Clin Chem Lab Med, 44*, 883-887 (2006). 14. Yushkevich *et al. Hum Brain Mapp*, 36, 258-287 (2015). 15. Woolley & McEwen. *J Comp Neurol. 336*, 293-306 (1993). 16. Bimonte-Nelson *et al. Neuroreport, 15,* 2659-2663 (2004). 17. Maass *et al. Elife*, *4*, e06426 (2015). 18. Braak & Braak. *Acta neuropathol*, *82*, 239-259 (1991). 19. Mazure & Swendsen. *Lancet Neurol*, *15*, 451 (2016).

Contact information: cmtaylor@ucsb.edu