Impact of Sex Steroid Hormones on Human Cerebellar Network Organization

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UCSB BRAIN INITIATIVE

INTRODUCTION

- **The cerebellum exhibits rich expression of sex steroid hormones and receptors.**
  - Across a typical menstrual cycle (~28 days), the average female will experience a 12-fold increase in estrogen and an 800-fold increase in progesterone[1].
  - Previous work demonstrates that estradiol shapes functional connectivity of the cerebral cortex across the cycle[2].
- **Current study: How do sex steroid hormones impact resting-state functional connectivity of the cerebellum?**
  - Utilized the 28andMe dataset[2] to examine the extent to which endogenous fluctuations in estradiol and progesterone across a complete menstrual cycle alter functional connectivity of cerebellar networks at rest.

METHODS

PARTICIPANT: The participant 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: The participant underwent daily time-locked (±30 min) 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.

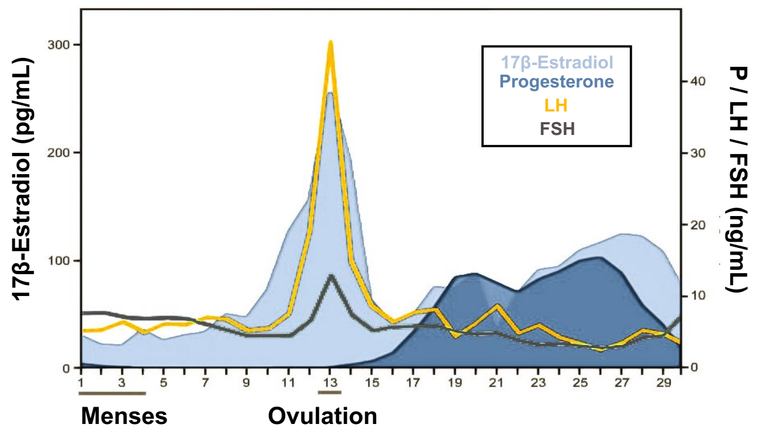
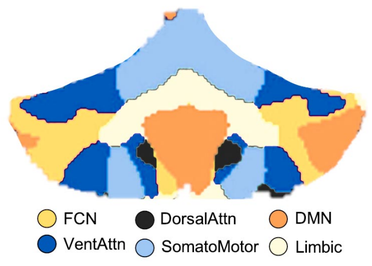


Figure 1. Participant's hormone concentrations plotted by day of cycle. 17β-estradiol, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels fell within standard ranges. Adapted from Pritschet et al. 2020.

MRI PROCESSING: We acquired a daily 10 min. resting-state scan on a 3T Siemens Prisma at the UCSB Brain Imaging Center (T2* multi-band EPI; 72 oblique slices; TR = 720 ms; voxel size = 2 mm^3). Data were realigned/unwarped, registered to a subject-specific anatomical template (created with ANTs), and smoothed (4 mm FWHM) in SPM12; in-house Matlab scripts were used for additional preprocessing, including global scaling, detrending, nuisance regression, and temporal filtering using a maximal overlap discrete wavelet transform. Signal from the adjacent left/right cerebral cortex within 7 mm of the cerebellum was regressed out.

RESTING-STATE FUNCTIONAL CONNECTIVITY (RSFC) ANALYSES:

7-network parcellation



For each day, eigen-timeseries from 99 clusters defined by the Ren[3] atlas were extracted and assigned to 7 functional networks identified by Buckner et al[4]. Edgewise functional connectivity was estimated via magnitude squared coherence, restricted to low-frequency fluctuations in wavelet scales 3-6 (~0.01 - 0.17 Hz). All association matrices were FDR-thresholded (q < .05). Common graph theory metrics were employed to characterize functional network topology: **efficiency** (a measure of *within* network integration) and **participation coefficient** (a measure of *between* network integration)[5]. Spearman's correlation was used to address associations between hormones and network topologies, while vector autoregressive models evaluated time-lagged effects.

CONCLUSIONS & FUTURE AIMS

- Serum concentrations of E and P fell within expected ranges[6] and displayed the canonical fluctuations across the menstrual cycle, with E peaking in late follicular phase and P concentrations rising dramatically during the mid-luteal phase.
- **Time-synchronous edgewise analyses: Increases in progesterone over time are associated with robust decreases in functional connectivity across the cerebellum.**
- **Time-synchronous correlational analyses: Increases in estradiol over the cycle are correlated to decreases in functional connectivity across the cerebellum.**
- **Time-Lagged analyses: Networks exhibit functional stability** among metrics of between- and within-network integration.
- Daily hormone levels demonstrate **negative** associations with cerebellar connectivity, while temporal variability in the cerebellum is attributed *more* to previous network states rather than estradiol concentrations.

References
1. Stricker *et al. Clin Chem Lab Med. 44*, 883-887 (2006) 2. Pritschet *et al.* (2020). 3. Ren *et al. Scientific reports,* 9(1), 1-12 (2019). 4. Buckner *et al. Journal of neurophysiology,* 106(5), 2322-2345 (2011). 5. Bullmore et al. *Annu. Rev. Clin. Psychol. 7,* 113-140 (2011). 6. Fehring et al. *J Obstet Gynecol Neonatal Nurs. 35(3),* 376-384 (2006).

Acknowledgments
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RESULTS

Time-Synchronous Analysis: Edgewise Regression

Increases in progesterone and estradiol across the cycle are associated with ***diminished*** **cerebellar functional connectivity**

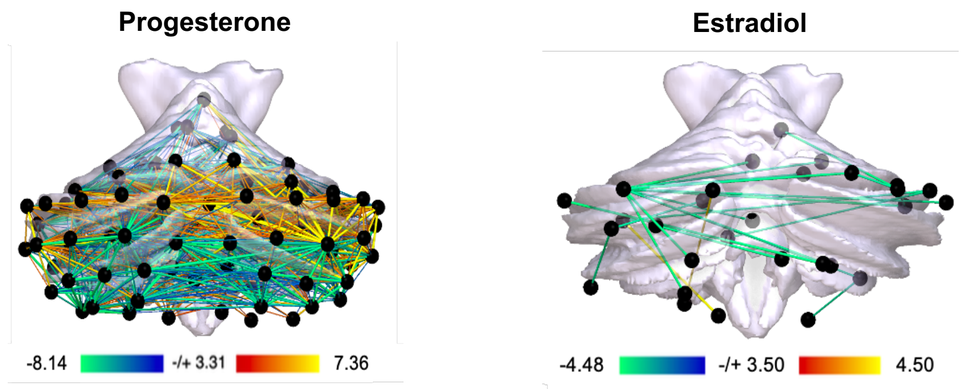


Figure 2. Edgewise regression between coherence and hormones. 'Cooler' colors indicate decreasing coherence with increasing hormone concentrations (FDR-corrected, q < .05).

Time-Synchronous Analysis: Correlation

Increases in estradiol across the cycle are correlated with **decreases in global efficiency**

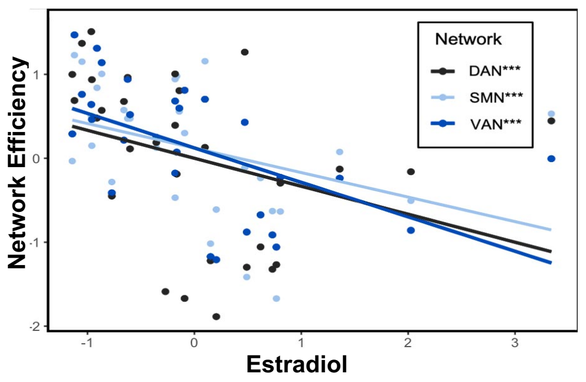


Figure 3. Time-synchronous associations between network efficiency and estradiol. Spearman's correlation between global efficiency and estradiol (FDR-corrected at q < .05; '***' indicates p < .001). SMN and VAN efficiency were greater than three standard deviations from the mean on day 21 of cycle and were removed from the plot. Abbreviations: DAN, Dorsal Attention Network; SMN, Somato-Motor Network; VAN, Ventral Attention Network.

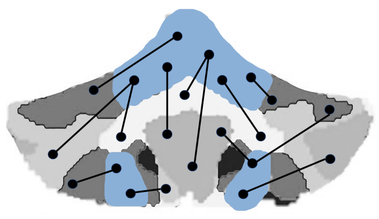
Time-Lagged Analysis: Vector Autoregression

In order to more directly capture time-dependent modulation of network connectivity and hormonal states, we specified and estimated simultaneous 2nd-order vector autoregressive models:

$$\mathbf{Brain}_t = \mathbf{Brain}_{t-1} + \mathbf{Estradiol}_{t-1} + \mathbf{Brain}_{t-2} + \mathbf{Estradiol}_{t-2}$$
$$\mathbf{Estradiol}_t = \mathbf{Brain}_{t-1} + \mathbf{Estradiol}_{t-1} + \mathbf{Brain}_{t-2} + \mathbf{Estradiol}_{t-2}$$

BETWEEN-NETWORK CONNECTIVITY

- Estradiol did not predict between-network connectivity.
- Cerebellar **DMN** and **SMN** exhibited significant autoregressive effects of connectivity at lag 1 but lacked significant overall model fits.



$Estradiol_t = SMN_{t-1} + Estradiol_{t-1} + SMN_{t-2} + Estradiol_{t-2}$

Term	Est	*SE*	*T(p)*
SMN_{t-1}	**0.44**	**0.21**	**2.12 (.041)**
$Estro_{t-1}$	0.12	0.24	0.48 (.631)
SMN_{t-2}	0.15	0.19	0.79 (.442)
$Estro_{t-2}$	-0.17	0.24	-0.70 (.491)
R^2 = 0.27 (p = .099); RMSE = 0.78 (p = .035)			

$Estradiol_t = DMN_{t-1} + Estradiol_{t-1} + DMN_{t-2} + Estradiol_{t-2}$

Term	Est	*SE*	*T(p)*
DMN_{t-1}	**-0.34**	**0.17**	**-1.98 (.046)**
$Estradiol_{t-1}$	-0.28	0.19	-1.46 (.155)
DMN_{t-2}	-0.09	0.13	-0.68 (.496)
$Estradiol_{t-2}$	0.08	0.19	0.42 (.666)
R^2 = 0.21 (p = .189); RMSE = 0.62 (p = .004)			

WITHIN-NETWORK CONNECTIVITY

- Estradiol did not predict within-network connectivity.
- Cerebellar **DAN** (lag 1), **VAN** (lag 2), and **SMN** (lag 2) exhibited significant autoregressive effects of network states, with significant model fits for the **DAN** and **VAN**.

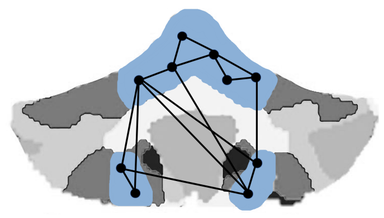


Diagram of DAN model fit: efficiency

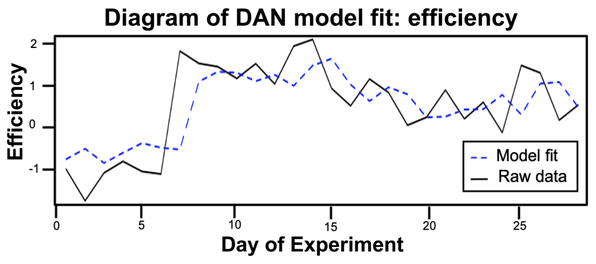
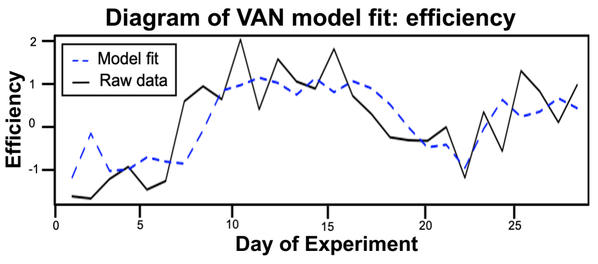


Diagram of VAN model fit: efficiency



$Estradiol_t = DAN_{t-1} + Estradiol_{t-1} + DAN_{t-2} + Estradiol_{t-2}$

Term	Est	*SE*	*T(p)*
DAN_{t-1}	**0.55**	**0.20**	**2.68 (.014)**
$Estradiol_{t-1}$	-0.11	0.21	-0.54 (.596)
DAN_{t-2}	0.11	0.20	0.58 (.577)
$Estradiol_{t-2}$	0.07	0.20	0.33 (.746)
R^2 = 0.50 (p = .002); RMSE = 0.65 (p = .0003)			

$Estradiol_t = VAN_{t-1} + Estradiol_{t-1} + VAN_{t-2} + Estradiol_{t-2}$

Term	Est	*SE*	*T(p)*
VAN_{t-1}	0.33	0.18	1.89 (.052)
$Estradiol_{t-1}$	-0.30	0.17	-1.78 (.088)
VAN_{t-2}	**0.33**	**0.12**	**2.82 (.004)**
$Estradiol_{t-2}$	0.24	0.17	1.42 (.163)
R^2 = 0.52 (p = .002); RMSE = 0.52 (p = .0002)			