Menstruation and Visual Perceptual Learning

By
Hana Berhe
April 20, 2024
Brown University, Providence, RI

Thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Arts with Honors in Health and Human Biology

Mentors: Yuka Sasaki, Takashi Yamada
Second reader: Takeo Watanabe

Abstract:

Visual Perceptual Learning (VPL) is the ability to detect, discriminate, or identify visual information through training over a long period of time. While sleep has been established as a crucial contributor to VPL consolidation, the influence of fluctuating menstrual cycle phases on this process remains largely unexplored. This study looks at the association between menstrual cycle phases and VPL performance gains in women with normal menstrual cycles, who have no history of neurological disorders. Participants completed a visual perception task and were exposed to EEG and MRS during various menstrual phases. Fast sigma wave activity in sleep and E/I neurotransmitter ratios in the visual and prefrontal cortices were evaluated as markers of VPL consolidation.

Results showed a decrease in fast sigma power and a decrease in E/I ratios during non-REM sleep for women in menses compared to women in other menstrual phases. This suggests a decrease in neuroplasticity and reduced learning facilitation. Correspondingly, offline gains were also reduced for women in menses. The findings suggest that menstrual cycle variations significantly influence sleep dependent consolidation of VPL, where menses is associated with decreased neuroplasticity, and reduced learning. These variations have implications for the optimization of visual training regimens for women.

Introduction:

Visual perceptual learning (VPL) is the long-term improvement of detection, discrimination, or identification of visual information through training (1). VPL also exhibits the neural functions of plasticity. However, the processes behind VPL remain largely in question, and discrepancies in the extent of VPL in different individuals also continue to be elusive (2).

The importance of sleep in VPL has been identified, where non-resting eye movement (NREM) sleep is significant in expanding neuroplasticity and resting eye movement (REM) sleep is significant in the consolidation of VPL (3). Neuroplasticity can be defined as the structural and functional modification of the nervous system that allows for alterations in neural activity and learning (13). There is also evidence of performance gains occurring either after overnight sleep (4,5) or daytime naps (6). It is also important to note that deprivation of

overnight, REM, or slow-wave (sleep stage N3) sleep, results in reduced VPL performance gains (5,7,8). VPL performance gains are not associated with only the passage of time between training and testing, which indicates that sleep is critical in VPL (7,8,9),.

There are also numerous parameters that have been used to measure changes in learning during sleep. Electroencephalogram (EEG) tests use electrodes to identify neural wave patterns. Different neural wave patterns are associated with different neural functions. The EEG waves that correspond to a frequency of 12-16 Hz are fast sigma waves (31), and are related to learning during sleep. Specifically, a positive correlation has been found between fast sigma waves during sleep stage 2 and IQ in healthy adults, whereas a negative correlation was found for children with ADHD (30). These results have indicated that there is a relationship between fast sigma power during stage 2 sleep and cognitive performance (30).

In addition to the use of EEG technology to measure sleep parameters, VPL studies also use magnetic resonance spectroscopy (MRS). MRS data has led to the discovery that the ratio of glutamate to GABA indicates the degree of neural plasticity (40). Moreover, studies have found a negative correlation between neuroplasticity (as it relates to motor learning) and GABA concentrations (41,42), while a positive correlation between glutamate and neuroplasticity has been detected (43). Excitatory (Glutamate + Glutamine) to inhibitory (GABA) ratios have also been linked to varying degrees of VPL. Increased excitatory/inhibitory (E/I) balance during non REM (NREM) sleep is associated with increased performance gains, while decreased E/I balance during REM sleep is associated with stabilization of learning. This has encouraged researchers to deduce that NREM sleep increases neuroplasticity to encourage learning while REM sleep reduces plasticity to promote stabilization of that learning (44,45). Previous studies focused on early visual areas, specifically the posterior part of the occipital lobe (45).

Although E/I balance was previously tested in the occipital lobe, other regions of interest could be significant indicators of VPL through E/I balance as well. The prefrontal cortex for example, has been shown to be significant in information filtering and the formation of cognitive maps (53,54). The ventral medial prefrontal cortex (vmPFC) specifically, has been found to relate with individuals' abilities to direct attention to specific information and to their degree of learning (55). The dorsolateral prefrontal cortex (dlPFC) is also associated with learning and memory (56). Hence, these regions were examined closely for this study.

VPL has many parameters that can indicate the degree of learning, however, influences on VPL continue to be discovered. This study seeks to identify if menstrual cycles are impactful on VPL. Menstrual cycles have variable durations which average about 28 days (16). The cycle begins with the follicular phase (days 1-14), during which primarily estrogen (specifically 17-beta-estradiol) is increased due to the upregulation of Follicle Stimulating Hormone (FSH) receptors (17). This phase is essential to the growth of the endometrial lining for potential implantation by sperm cells as well as the creation of channels in the cervix that provide suitable conditions for sperm cells to survive (16).

The second phase is ovulation (typically on day 14) which involves high levels of estrogen which functions as a positive feedback hormone to increase FSH and Luteinizing Hormone (LH) levels (17). This surge of LH results in the release of a mature oocyte due to the breakdown of the follicle, alterations in cervical mucus to continue supporting the passage of sperm and the eventual drop of estrogen levels at the end of ovulation (17).

Phase three of the menstrual cycle is labeled the Luteal or secretory phase (day 14-28). During this phase, progesterone levels (stimulated by the surge of LH) heighten. The mucous layer in the cervix thickens and body temperature tends to increase due to increased hypothalamic temperature. Hence, the endometrium prepares for potential implantation, and if implantation by sperm does not occur, the corpus luteum regresses. In addition, progesterone functions in negative feedback, resulting in a rapid decline in hormone levels proceeding the luteal phase (17).

The final phase of the menstrual cycle is menses where the buildup of endometrial lining sheds due to the fall in hormone levels after the luteal phase. Menses typically occur between day zero and day five of the upcoming menstrual cycle (17).

Differences in menstrual cycle phases have been shown to influence learning through neuroplasticity (10,11,12). Furthermore, a relationship between menstrual cycle phases and learning has been identified through gains in motor learning, which indicates that the Luteal phase of the menstrual cycle has reduced performance gains in motor learning as compared to the ovulation phase (14). However, the influence of the menstrual cycle phase on sleep-consolidated VPL is largely uncovered. There has also been evidence suggesting there is a strong relationship between menstrual cycle phase and memory consolidation (15). Mense's or

‘perimenses’ (meaning around the time of, or during menses), was associated with reduced performance, and lowered memory retention (15).

Menstrual cycle phase changes have also been associated with different sleep parameters. Women have self-reported reduced sleep quality during pre-menstruation and menstruation (menses) (38,39). In addition, some studies found that REM sleep is reduced in women in the mid-luteal phase as compared to women in their mid-follicular phase (36). Some studies hypothesize that reduced REM sleep during the luteal phase could be due to raised body temperatures (37).

The present study aims to investigate the relationship between VPL and differing menstrual cycle phases. Women with no history of neurological disorders experiencing different menstrual cycle phases were asked to complete a questionnaire, and were studied using EEG and MRS to identify discrepancies in E/I ratios and fast sigma waves, to identify if these neurological conditions are associated with different offline gains and performance. We hypothesized that the mense phase would have reduced offline gains due to the reduced sleep quality reported by women (38,39), and the influence of sleep on consolidation of learning. We also hypothesize that during menses, E/I ratios are lower during NREM sleep and higher for REM sleep since these are correspond to lowered VPL. Overall we hypothesize that VPL is reduced during menses.

Methods:

Participants:

16 healthy females with no record of pre-existing neurological disorders were recruited. The participants reported regular sleep schedules with 2-hour deviance when comparing their weekday versus weekend sleep schedules. The average regular duration of sleep for the participants was reported to be between 6 and 9 hours. The participants were asked to complete a sleep-wake questionnaire

Screening Process for Determining Eligibility:

Subjects were screened prior to being provided consent forms. Participants were required to be between the ages of 18 and 30. Participants also had corrected-to-normal or normal vision.

Subjects underwent a sleep-wake habits questionnaire (18, 19) to determine if they had normal sleep habits. They also completed the Munich Chronotype Questionnaire (MCTQ) to understand the subject's individual sleep-wake rhythms on weekdays and weekends. Using information from these questionnaires, participants were only included in the study if they have no sleep-related problems and if their wake-sleep schedules differed by no more than 2 hours on weekdays and weekends. Participants also had sleep durations which averaged between 6 and 9 hours daily. Subjects who had previously participated in VPL studies were also deemed ineligible, to reduce confounding variables. Participants were also required to complete a video game questionnaire (18,19, 20), as excessive video game playing -beyond 5 hours a week within the last 6 months- has been associated with visual processing ability (20, 21).

Due to the self-report nature of the screening process, there is a possibility that some participants reported having no sleep problems although they experience otherwise. Thus, participants with irregular sleep structure as recorded in this study (such as participants with irregular sleep onset REM sleep periods), hence, the sleep patterns of our participants were determined to be within normal limits.

Experimental design:

The experimental design for this study was largely modeled by a previous study outlining the influence of REM and non-REM sleep on VPL (23). This study included two training sessions separated by a 120-minute interval. The training sessions were conducted on a texture discrimination task described below.

The 120-minute interval included a 90-minute nap and a 30-minute break. The break was designed to reduce the negative impacts of sleep inertia (22). During the break, participants underwent a questionnaire designed to reveal individual perceptions of sleep onset latency (SOL), wake-time after sleep onset (WASO), the occurrence of dreams, and comfort of the environment (23).

All participants underwent a sleep session designed for adaptation to prevent a significant influence of the first night effect (FNE) as a result of sleeping in a foreign environment (24). During the adaptation session, subjects were placed in the same conditions they would experience during the sleep sessions post-training. Polysomnography (PSG) recording technology was integrated during this sleep session as well, in order to mimic proceeding sleep

sessions . Sleep sessions with training were conducted a week post-adaptation session in order to resist the influence of the adaptation sleep session on the participants' sleep patterns (23).

Due to a reduced number of participants, this study conducted 2 sleep sessions for seven participants, and 3 sleep sessions for nine of the participants. Both groups of participants were only trained and given TDT tasks during their final sleep session, in order to ensure prior learning did not influence performance gains. We excluded data from the first sleep session due to the influence of the FNE. We used data from sleep sessions involving training.

Three test sessions were conducted. The first session was before the training of TDT-A, the second test was after the nap, and the third was after the training of TDT-B. After the first test session, we attached the electrodes for PSG. The MRI noise was then turned off, and participants were then placed in the MRI, where we conducted voxel placement to specify the location of the neurotransmitter we sought to detect (voxel was placed at the dorsolateral prefrontal cortex (dlPFC) and ventral-medial prefrontal cortex (vmPFC)), anatomical structure measurement, and shimming to optimize MRI scanner homogeneity. The voxel was 25mm by 25mm by 25mm. The room lights were then turned off and participants underwent their sleep sessions while MRS data was collected (the sleep session ran for about 90 minutes).

Texture discrimination task:

The TDT used in this study was modeled after a standard VPL task often utilized to understand the influence of sleep on VPL (5, 23, 25, 26, 27). The stimuli used in this task were developed using the software, MATLAB with the psychtoolbox (23, 28, 29). The participants' chin was placed on a chin rest, in order to control for visual angle. Controlling for visual angle was critical, as shifts in visual angle can lead to extinguished performance gains (32,33,34).

The texture determination tasks (TDTs) included a background that was orthogonal to the pre-sleep (TDT-A) and post-sleep (TDT-B) meaning that the textures were at right angles to each other (horizontal or vertical). The reasoning behind using orthogonal textures is that these orientations have been found to incur retrograde interference (35). However, REM sleep helps oppose this retrograde interference, indicating that sleep is necessary to stabilize learning (23). This allows us to specifically test for sleep consolidated VPL.

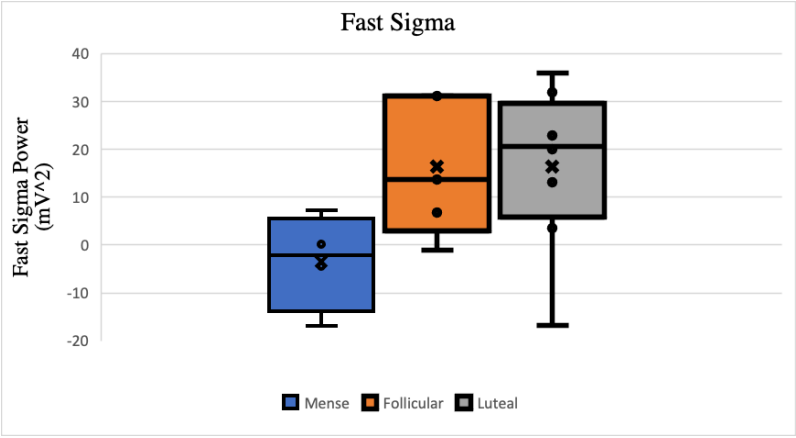
Results:

Do altering menstrual cycle phases influence fast sigma patterns, E/I balance, and offline gains? Our hypothesis is that the mense phase of the menstrual cycle will have reduced fast sigma patterns, reduced offline gains, lower NREM E/I ratios, and increased REM E/I ratio as compared to other menstrual cycle phases.

Data was taken from sleep sessions 2 and 3. Participants from sleep session 2 had the first session as the adaptation session in order to eliminate the first night effect (FNE) and the second sleep session included training. Participants from sleep session 3 had also had the first session focused on adaptation, but had a second sleep session without learning, and finally participated in training for sleep session 3. In this sense, this study equates the trained sleep session 2 participants with trained sleep session 3 participants, as the FNE was eliminated for both of these groups. Data was collected from 16 participants total (n=16).

The alpha (α) level determined for the results was 0.05 and was set for the statistical analysis. It remains critical to consider the reduced number of participants in this study, as strong relationships between data (although not statistically significant) could be indicative of practical relevance. One-sided t-tests were conducted with the different sleep parameters for which evidence based hypotheses were formulated.

Fast Sigma Waves



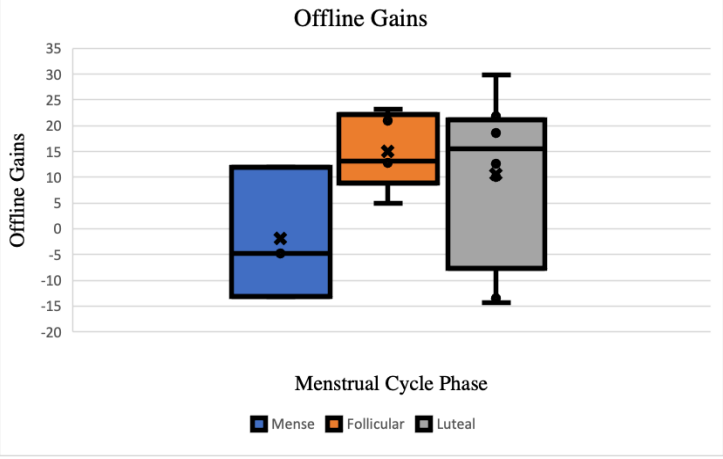
All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data. The mean for mense (as represented by the 'X' point) is -4.8126, the median (represented by the median line) is -4.5560, the maximum value (represented by the upper bar) is 7.1438 whereas the minimim value (represented by the lower bar) is -17.026. For follicular, the mean is 16.391, the median is 13.725, the maximum is 31.227 and the minimum is -1.0405. For luteal, the mean is 16.469, the median is 20.640, the maximum is 31.834 and the minimum is -16.722.

Due to relationships identified within previous studies between fast sigma waves and learning (30), this parameter was studied. Fast sigma

waves were measured as fast sigma wave power in the trained occipital region minus fast sigma wave power in the untrained occipital region. This was designed in order to account for the individual variations in fast sigma power, allowing us to control for neurological diversity.

Our study tested for fast sigma waves during stage 2 of sleep (for more details refer to reference table 1). Fast sigma power in individuals in the mense phase was significantly reduced as compared to the follicular phase (two-sample and one-sided t-test, n=8 subjects, degrees of freedom = 6, t_6 = -2.1125, p=0.0396). For menses as compared to the luteal phase, there was also a significant reduction in sigma power (two-sample and one-sided t-test, n=11 subjects, degrees of freedom = 9, t_9 = -1.9825, p=0.0394). When comparing sigma waves in participants in the luteal and follicular phase, we did not have a hypothesis suggesting increased sigma waves in either menstrual cycle phase, thus, we conducted a two-sided two test, that revealed no statistically significant results (two-sample and two-sided t-test, n=13 subjects, degrees of freedom = 11, t_{11} = -0.0085, p=0.9934).

Offline Gains



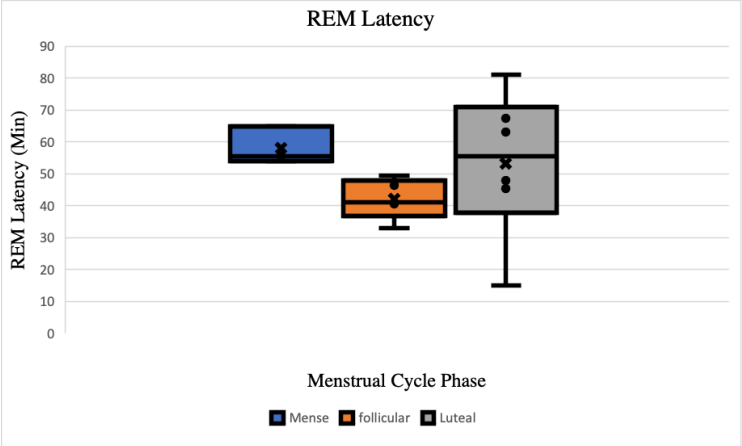
All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data. The mean for mense (as represented by the 'X' point) is -1.9505, the median (represented by the median line) is -4.7521, the maximum value (represented by the upper bar) is 11.985 whereas the minimim value (represented by the lower bar) is -13.086. For follicular, the mean is 15.007, the median is 13.094, the maximum is 23.255 and the minimum is -4.9449. For luteal, the mean is 10.535, the median is 15.573, the maximum is 29.860 and the minimum is -14.367.

The offline gains for the TDT were tested to determine if performance differed based on the menstrual phase individuals were in (review reference table 2 for more details). Offline performance gains were determined as improvements in TDT-A before and after sleep. Specifically, $gains = \frac{performance\ in\ session\ 3 - performance\ in\ session\ 2}{performance\ in\ session\ 2} * 100\%$. The only test sessions involved in gathering offline gains results were test sessions 1 and 2, and this parameter

analyzes changes in performance without further training (excludes TDT-B). We conducted one-sided t-tests for data regarding the menses phase, as we hypothesized based off of previous findings that women in menses would have reduced offline gains. However, we used two-sided t-tests for results regarding women only in the follicular and luteal phases.

The study found that compared to women in the follicular phase, women in menses had significantly reduced offline gains (two-sample and one-sided t-test, n=8 subjects, degrees of freedom = 6, t_6 = -2.4477, p=0.0250). Women in menses had reduced offline gains compared to women in the luteal phase as well, although these results are not statistically significant (two-sample and one-sided t-test, n=11 subjects, degrees of freedom = 9, t_9 = -1.1873, p=0.1327). Women in the luteal phase and the follicular phase had no significant or large differences in offline performance gains (two-sample and two-sided t-test, n=13 subjects, degrees of freedom = 11, t_{11} = 0.5733, p=0.5780).

REM Latency

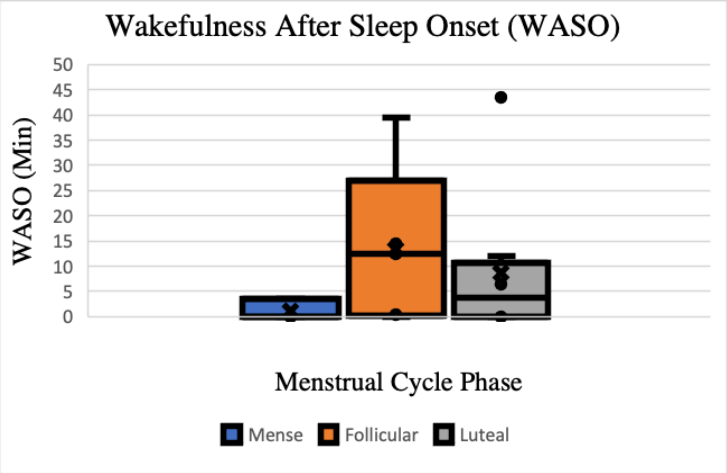


All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data. The mean for mense (as represented by the 'X' point) is 58.167 minutes, the median (represented by the median line) is 55.500, the maximum value (represented by the upper bar) is 65.000 whereas the minimim value (represented by the lower bar) is 54.000. For follicular, the mean is 42.100, the median is 41.000, the maximum is 49.500 and the minimum is 33.000. For luteal, the mean is 53.333, the median is 55.500, the maximum is 81.000 and the minimum is 15.000.

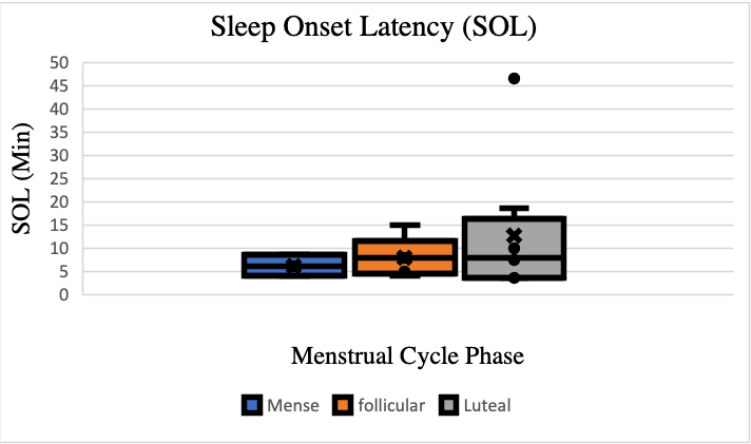
The results for REM latency (REML) were critical in our understanding of the influences of sleep on learning (refer to reference table 3 for more details). Due to the role of REM sleep in memory consolidation and learning (44,45), this sleep variable was included in the study design. However, although studies have shown that the amount of REM sleep varies between the follicular and luteal phase, REML is a variable for which we did not have a hypothesis. Thus, this component of study used a two sided t-test to analyze results. A significant difference in REML between the menses and follicular phase was found (two-sample and two-sided t-test, n=8

subjects, degrees of freedom = 6, t_6 = 3.5390, p=0.0122), and women in menses had a higher REML than those in the follicular phase. Women in menses and luteal phases had no significant differences in REML (two-sample and two-sided t-test, n=9 subjects, degrees of freedom = 7, t_7 = 0.3487, p=0.7376), and women in luteal and follicular phases had no significant differences in REML (two-sample and two-sided t-test, n=11 subjects, degrees of freedom = 9, t_9 = -1.0556, p=0.3187).

Sleep Onset Latency (SOL) and Wakefulness After Sleep Onset (WASO)



All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data. The mean for mense (as represented by the 'X' point) is 1.6667 minutes, the median (represented by the median line) is 0.0000, the maximum value (represented by the upper bar) is 3.5 whereas the minimim value (represented by the lower bar) is 0.0000. For follicular, the mean is 13.400, the median is 12.500, the maximum is 39.500 and the minimum is 0. For luteal, the mean is 8.7500, the median is 3.7500, the maximum is 12.000 and the minimum is 0.0000. There is an outlier for luteal data at 43.500 minutes.

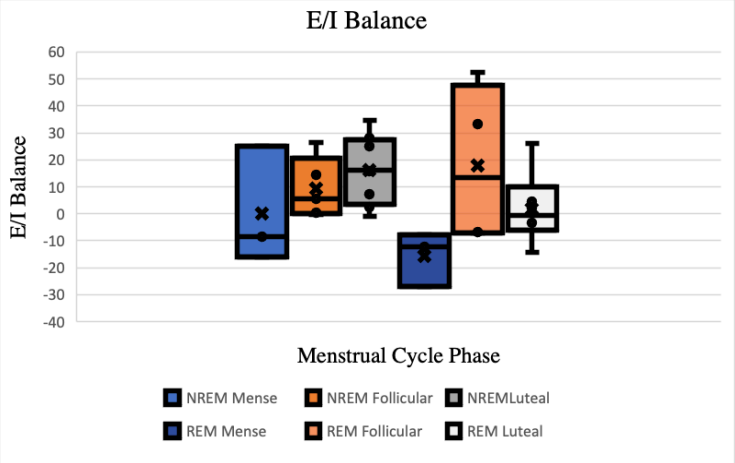


All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data. The mean for mense (as represented by the 'X' point) is 6.1667 minutes, the median (represented by the median line) is 6.0000, the maximum value (represented by the upper bar) is 8.5000 whereas the minimim value (represented by the lower bar) is 4.0000. For follicular, the mean is 8.0000, the median is 8.0000, the maximum is 15.000 and the minimum is 4.0000. For luteal, the mean is 12.750, the median is 8.0000, the maximum is 18.500 and the minimum is 4.0000. There is an outlier for luteal data at 46.500 minutes.

As women self-reported reduced sleep quality pre-menses and during menses, we hypothesized that SOL and WASO would be increased during this phase of the menstrual cycle. When comparing luteal and follicular phases we hypothesized that the luteal phase would have increased SOL and WASO due to the raise in body temperature during this phase. Thus, for these

results we utilized one sided t-tests, for increased SOL and WASO during menses and luteal phases. We also excluded outlier data values, which are represented in the graphs. The data is further detailed in reference table 4 and reference table 5. When comparing menses with the follicular (two-sample and one-sided t-test, n=8 subjects, degrees of freedom = 6, t_6 = -1.2740, p=0.8751) and menses with the luteal phase (two-sample and two-sided t-test, n=10 subjects, degrees of freedom = 8, t_8 = -0.8943, p=0.3973), there was no statistically significant relationship in WASO. When comparing the follicular and luteal phases, there were also no significant differences in WASO (two-sample and one-sided t-test, n=12 subjects, degrees of freedom = 10, t_{10} = 1.5212, p=0.9204). SOL for women in menses as compared to follicular (two-sample and one-sided t-test, n=8 subjects, degrees of freedom = 6, t_6 = -0.6703, p=0.7362) and luteal phases (two-sample and two-sided t-test, n=10 subjects, degrees of freedom = 8, t_8 = -0.5362, p=0.6064) had no significant differences. WASO comparing follicular and luteal phases also resulted in no significant difference (two-sample and one-sided t-test, n=12 subjects, degrees of freedom = 10, t_{10} = -0.0245, p=0.5096).

E/I Ratios



All boxes represent the interquartile range (IQR) for the different data points, and indicate the middle 50% of the data.
For REM sleep, the mean for mense (as represented by the 'X' point) is -15.661, the median (represented by the median line) is --46.984, the maximum value (represented by the upper bar) is -7.6536 whereas the minimim value (represented by the lower bar) is -26.921. For follicular, the mean is 17.982, the median is 13.333, the maximum is 52.318 and the minimum is -7.0546. For luteal, the mean is 1.9409, the median is -0.59700, the maximum is 26.245 and the minimum

E/I balance in this study represents changes in E/I balance after training. For example, negative values in our data (reference table 6) indicate reduced E/I balance during the specific sleep stage as compared to E/I ratios immediately after training. In this sense, we only measured E/I balance changes during sleep. Due to previous studies having found relationships between women in menses and reduced memory performance (15), we hypothesized that the E/I balance

will be lower during NREM sleep (as higher E/I balance during NREM sleep is associated with increased learning and we hypothesize reduced sleep consolidated learning) and and E/I balance changes for REM sleep will be greater for individuals in menses, as these are the ratios associated with reduced VPL (45). Thus, we used one sided t-tests (leaning towards menses having smaller E/I balance during NREM sleep than other phases) for all E/I ratio comparisons including menses during NREM sleep only. For REM sleep, we used a two sided t-test. A table of results for E/I ratios and their changes is listed below under reference table 6. E/I ratios between menses and the follicular phase had no significant differences during NREM sleep (two-sample and one-sided t-test, n=8 subjects, degrees of freedom = 6, t_6 = -0.8028, p=0.2263) and no significant differences in E/I balance during REM sleep (two-sample and two-sided t-test, n=7 subjects, degrees of freedom = 5, t_5 = -1.8453, p=0.1243), although a strong relationship between these variables exists where the E/I balance for REM sleep in menses is lower than follicular. E/I balance in NREM sleep, between menses and luteal phases had no statistically significant results although there was a strong relationship where NREM sleep had a lower E/I balance (two-sample and one-sided t-test, n=11 subjects, degrees of freedom = 9, t_9=-1.5418, p=0.0788), and no significant change in E/I ratio during REM (two-sample and one-sided t-test, n=9 subjects, degrees of freedom = 7, t_7 = -1.9678, p=0.0898), although there was a strong tendency for menses to have a lower E/I ratio.

Between the luteal and follicular phases we conducted two sided t-tests, and found no significant relationship in NREM sleep (two-sample and two-sided t-test, n=13 subjects, degrees of freedom = 11, t_{11} = 0.9694, p=0.3532) and no significant difference in E/I ratio change for REM sleep (two-sample and two-sided t-test, n=10 subjects, degrees of freedom = 8, t_8 = 1.1770, p=0.2730).

Discussion:

There was a clear reduction in parameters used to measure VPL in participants within the menses phase. Fast sigma power and offline gains were significantly reduced for women in menses (although not always statistically significant differences, these differences were prevalent considering the reduced sample size). In addition, E/I balance during NREM and REMsleep was

reduced for women in menses as compared to women in the luteal phase (although these results were not statistically significant, the small sample size permits this assumption). Although we did not hypothesize increased REML during menses as compared to the follicular phase, these results were found. The parameters that yielded no variations for different phases include, WASO and SOL which indicate processes not associated with the reduction in VPL seen in women during their menses phase.

The discrepancy in VPL (as determined by fast sigma power and offline gains, with larger values corresponding to more VPL) could be due to changes in brain size during the menstrual cycle (46). Gray matter and white matter compose neural tissue, and gray matter is found on the outer layers of the brain whereas white matter is found in inner layers. Gray matter is so named due to its gray tone that results from the high density of neuronal cell bodies found in this tissue (47). Neural gray matter peaks during ovulation which typically occurs during the follicular phase, however, the specific hormones that result in changes in gray matter cannot be deduced (46). In addition, gray matter has been found to relate to learning, specifically through the learning of specific skills (48). Gray matter volume was found to increase as participants were trained on a task, hence, increased learning is associated with more gray matter (48).

This could explain the enhanced performance of participants in the follicular phase as compared to menses, although it does not explain the lack of differences in performance for participants in the luteal phase as compared to follicular. However, the limited size of participants does not allow us to completely extinguish the possibility of a discrepancy in performance between the luteal and follicular phases. We hypothesize that rather than being a result of gray matter decrease, our findings could depict a potential cause of reductions in gray matter. Due to reduced learning during menses as compared to during the follicular phase for example, gray matter volume could have been reduced. Further research could be beneficial in understanding the specific reasons behind gray matter reductions in phases other than the follicular phase.

Another parameter that is critical to explore is sleep quality. Our findings suggest that there are no differences in sleep quality (as determined by SOL and WASO) for women in different menstrual phases. Due to other studies where women self-reported sleep disturbances during menstruation, we initially hypothesized increased values for SOL and WASO during this phase. This could have been due to anxiety during menses which has been found to be elevated,

and anxiety is also known to significantly affect sleep quality (49, 50, 51). Due to the relationship between sleep and learning (4, 5, 52), reduced sleep quality could suggest a mechanism through which menses influences VPL. Nonetheless, the sleep quality results were not significant, hence, our study does not support the conclusion that anxiety causing sleep disturbance influences learning.

Although sleep quality was invariable during different phases, REML was significantly increased in participants in menses as compared to the follicular phases. Increased REML and reduced performance were observed for women in menses, indicating a potential relationship between REML and decreased learning. Previous studies identified reduced REM sleep in women in their luteal phase as compared to women in their follicular phase (37). Our study focuses on REML hence, our results do not directly conflict with past findings. Previous studies have also shown that there is a relationship between reduced REM sleep and learning (3), but do not indicate the influence of REML on learning, hence, we propose further studies analyze this relationship.

In addition, E/I ratio data exhibits reduced values for NREM sleep as hypothesized. Previous findings depict that, during NREM sleep, there is an enhancement in the balance between E/I neural activity in the early visual regions, regardless of whether the individual had engaged in any learning activities before sleep (3). This particular elevation in the E/I ratio during NREM sleep was found to be connected with improved performance on a visual learning task after sleep, compared to their performance prior to sleep. This rise in the balance of E/I neural activity during NREM sleep is believed to signify an enhancement in the brain's ability to adapt and change, thereby promoting the process of learning (3, 23, 40, 41, 42, 43). Conversely, if the E/I balance during NREM sleep is reduced -as during menses- instead of increased, it could dull the usual NREM-related plasticity from occurring.

Our study also found that the E/I ratio during REM sleep was lower for menses than for other menstrual cycle phases. This counters our hypothesis, that lower REM sleep E/I ratios would be associated with menses due to the reduced gains observed during this phase. This indicates that rather than the influence of sleep-dependent consolidation of learning (which is greatly reliant on REM sleep), on offline gains for women in menses, the hindered performance is most likely due to variations in neuroplasticity. The constant reduction of E/I ratios through NREM and REM sleep could also suggest that the variations of E/I balance that are critical to

VPL are somewhat reduced for women in menses, and the models that have previously been proposed regarding increased E/I balance during NREM sleep, and decreased E/I balance during REM sleep may function alternatively for women in menses (3). We recommend further studies analyze E/I balance fluctuations in women in different menstrual cycle phases, and how these correspond to different neural functions.

A pharmacological study found that increasing GABA levels (reducing the E/I balance) significantly impaired procedural memory performance, potentially by tipping the E/I balance and decreasing plasticity during NREM sleep (57). Lower E/I balance values during NREM sleep, signify a state of reduced excitation relative to inhibition, which would be associated with diminished plasticity and less facilitation of learning. We suggest that reduced neuroplasticity during menses results in the hindrance of learning for women in this phase.

Limitations:

Several limitations of the present study should be acknowledged:

1. Individual Differences in VPL Abilities

Our study involved testing different women across menstrual cycle phases. Individuals can vary considerably in their baseline VPL abilities due to factors such as genetics, cognitive skills, and prior experience. This inter-individual variability in VPL capacities could have influenced the observed performance gains and neural correlates, independent of menstrual cycle effects. Future studies with larger sample sizes and measures of baseline VPL abilities would help disentangle these individual differences.

2. Sample Size

The study had a limited number of participants, which restricts the generalizability of the findings and the statistical power to detect smaller effect sizes. Replicating the results with a larger cohort would increase confidence in the reported effects of menstrual phases on VPL consolidation.

3. Novel Brain Regions Examined

While previous research has examined E/I neurotransmitter ratios in visual cortices for perceptual learning, this study explored E/I balance in the prefrontal cortex. However, the precise trends and functional significance of E/I ratios in these regions for VPL consolidation are not

well-established from prior work. As such, the observed E/I patterns in prefrontal areas during different menstrual phases should be interpreted with caution, as the normative ranges and implications for learning are currently unclear.

We recommend that more studies be conducted on the influence of the vmPFC and dlPFC on VPL and the functions of E/I ratios within these regions.

Appendix:

Reference Table 1:

Fast Sigma		
Mense	Follicular	Luteal
-4.5560062	31.2266316	20.1092902
7.14377277	6.84905732	35.9092255
-17.02564	13.725156	22.8611928
	31.1953156	-16.721965
	-1.0404753	21.1705135
		13.033629
		3.55316436
		31.8341079

Reference Table 2:

Offline Gains		
Mense	Follicular	Luteal
-4.7520599	4.94489432	29.859761
11.9852475	13.0942968	21.8117827
-13.084759	23.2546329	19.2616658
	20.9967347	-14.3665
	12.7442912	10.0962432
		12.5382531
		-13.528354

		18.6069292

Reference Table 3:

REM Latency (Minutes)		
Mense	follicular	Luteal
55.5	41	45.5
65	49.5	81
54	33	63
	40.5	67.5
	46.5	48
		15

Reference Table 4:

WASO (Minutes)		
Mense	Follicular	Luteal
0	39.5	7
3.5	0	1
0	0.5	0
	12.5	0
	14.5	12
		6.5
		0
		43.5

Reference Table 5:

SOL (Minutes)		
Mense	follicular	Luteal
6	8	3.5
4	4	3.5
8.5	8	8.5
	5	4
	15	7.5
		10
		46.5
		18.5

Reference Table 6:

E/I Balance								
NREM			REM			Change in E/I Balance		
Mense	Follicular	Luteal	Mense	Follicular	Luteal	Mense	Follicular	Luteal
-8.3744707	0.3803143	24.985818	-12.40845	-7.0545899	-3.4200708	-4.0339793	-7.4349042	-28.40590
-16.026719	-0.1235112	34.622391	-7.6535673	33.315589	-14.399208	8.3731517	33.439101	-49.02152
24.985818	26.413174	16.126494	-26.92149	52.317634	-3.1721934	-51.90732	25.904457	-19.29868
	14.51826	15.84326		N/A	26.24520		N/A	10.40193

	22	13			12			99
	5.43079674	2.35935948		-6.6490893	1.97818198		-12.07988604	-0.3811775
		7.16415829			N/A			N/A
		-0.883072			4.41353693			5.29660893
		28.1533087			N/A			N/A

References:

1. Awada, A., Bakhtiari, S., & Pack, C. C. (2021). Visual perceptual learning generalizes to untrained effectors. *Journal of vision*, *21*(3), 10. [PubMed]
2. Shibata, K., Sasaki, Y., Kawato, M., & Watanabe, T. (2016). Neuroimaging Evidence for 2 Types of Plasticity in Association with Visual Perceptual Learning. *Cerebral cortex (New York, N.Y. : 1991)*, *26*(9), 3681–3689. [PubMed]
3. Tamaki, M., & Sasaki, Y. (2022). Sleep-Dependent Facilitation of Visual Perceptual Learning Is Consistent with a Learning-Dependent Model. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *42*(9), 1777–1790. [PubMed]
4. Mascetti, L., Muto, V., Matarazzo, L., Foret, A., Ziegler, E., Albouy, G., Sterpenich, V., Schmidt, C., Degueldre, C., Leclercq, Y., Phillips, C., Luxen, A., Vandewalle, G., Vogels, R., Maquet, P., & Balteau, E. (2013). The impact of visual perceptual learning on sleep and local slow-wave initiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(8), 3323–3331. [PubMed]
5. Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., & Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science (New York, N.Y.)*, *265*(5172), 679–682. [PubMed]
6. Mednick, S., Nakayama, K., & Stickgold, R. (2003). Sleep-dependent learning: a nap is as good as a night. *Nature neuroscience*, *6*(7), 697–698. [PubMed]
7. Stickgold, R., James, L., & Hobson, J. A. (2000). Visual discrimination learning requires sleep after training. *Nature neuroscience*, *3*(12), 1237–1238. [PubMed]
8. Gais S., Plihal W., Wagner U., Born J., Early sleep triggers memory for early visual discrimination skills. Nat. Neurosci. 3, 1335–1339 (2000). [PubMed]
9. Walker M. P., Stickgold R., Sleep, memory, and plasticity. Annu. Rev. Psychol. 57, 139–166 (2006). [PubMed]
10. Maguire J., Mody I. Steroid Hormone Fluctuations and Gaba(a)R Plasticity. *Psychoneuroendocrinology.* 2009;34:S84–S90. [PubMed]
11. Finocchi C., Ferrari M. Female Reproductive Steroids and Neuronal Excitability. *Neurol. Sci.* 2011;32:S31–S35. doi: 10.1007/s10072-011-0532-5. [PubMed]

12. Catenaccio E., Mu W., Lipton M.L. Estrogen- and Progesterone-Mediated Structural Neuroplasticity in Women: Evidence from Neuroimaging. *Brain Struct. Funct.* 2016;221:3845–3867. [PubMed]
13. Von Bernhardi R., Bernhardi L.E., Eugenin J. What Is Neural Plasticity? *Adv. Exp. Med. Biol.* 2017;1015:1–15. [PubMed]
14. Ikarashi, K., Sato, D., Iguchi, K., Baba, Y., & Yamashiro, K. (2020). Menstrual Cycle Modulates Motor Learning and Memory Consolidation in Humans. *Brain sciences*, *10*(10), 696. [PubMed]
15. Sattari, N., McDevitt, E. A., Panas, D., Niknazar, M., Ahmadi, M., Naji, M., Baker, F. C., & Mednick, S. C. (2017). The effect of sex and menstrual phase on memory formation during a nap. *Neurobiology of learning and memory*, *145*, 119–128. [PubMed]
16. Rosner, J., Samardzic, T., & Sarao, M. S. (2022). Physiology, Female Reproduction. In *StatPearls*. StatPearls Publishing. [PubMed]
17. Thiyagarajan, D. K., Basit, H., & Jeanmonod, R. (2022). Physiology, Menstrual Cycle. In *StatPearls*. StatPearls Publishing. [PubMed]
18. Tamaki M, Wang Z, Watanabe T & Sasaki Y Trained-feature-specific offline learning by sleep in an orientation detection task. *J Vis* 19, 12 (2019). [PubMed]
19. Tamaki M, et al. Reward does not facilitate visual perceptual learning until sleep occurs. *Proc Natl Acad Sci U S A* 117, 959–968 (2020). [PubMed]
20. Berard AV, Cain MS, Watanabe T & Sasaki Y Frequent video game players resist perceptual interference. *PLoS One* 10, e0120011 (2015). [PubMed]
21. Green CS & Bavelier D Action video game modifies visual selective attention. *Nature* 423, 534–537 (2003). [PubMed]
22. Lubin A, Hord DJ, Tracy ML & Johnson LC Effects of exercise, bedrest and napping on performance decrement during 40 hours. *Psychophysiology* 13, 334–339 (1976). [PubMed]
23. Tamaki, M., Wang, Z., Barnes-Diana, T., Guo, D., Berard, A. V., Walsh, E., Watanabe, T., & Sasaki, Y. (2020). Complementary contributions of non-REM and REM sleep to visual learning. *Nature neuroscience*, *23*(9), 1150–1156. [PubMed]

24. Tamaki M, Bang JW, Watanabe T & Sasaki Y The first-night effect suppresses the strength of slow-wave activity originating in the visual areas during sleep. *Vision Res* 99, 154–161 (2014). [PubMed]
25. Yotsumoto Y, et al. Location-specific cortical activation changes during sleep after training for perceptual learning. *Curr Biol* 19, 1278–1282 (2009). [PubMed]
26. Schwartz S, Maquet P & Frith C Neural correlates of perceptual learning: a functional MRI study of visual texture discrimination. *Proc Natl Acad Sci U S A* 99, 17137–17142 (2002). [PubMed]
27. Bang JW, Khalilzadeh O, Hamalainen M, Watanabe T & Sasaki Y Location specific sleep spindle activity in the early visual areas and perceptual learning. *Vision Res* 99, 162–171 (2014). [PubMed]
28. Pelli DG The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spatial vision* 10, 437–442 (1997). [PubMed]
29. Brainard DH The Psychophysics Toolbox. *Spatial vision* 10, 433–436 (1997). [PubMed]
30. Bestmann, A., Conzelmann, A., Baving, L., & Prehn-Kristensen, A. (2019). Associations between cognitive performance and sigma power during sleep in children with attention-deficit/hyperactivity disorder, healthy children, and healthy adults. *PloS one*, *14*(10), e0224166. [PubMed]
31. Nayak, C. S., & Anilkumar, A. C. (2023). EEG Normal Waveforms. In *StatPearls*. StatPearls Publishing. [PubMed]
32. Sasaki, Y., Nanez, J. E., & Watanabe, T. (2010). Advances in visual perceptual learning and plasticity. *Nature reviews. Neuroscience*, *11*(1), 53–60. [PubMed]
33. Poggio, T., Fahle, M., & Edelman, S. (1992). Fast perceptual learning in visual hyperacuity. *Science (New York, N.Y.)*, *256*(5059), 1018–1021. [PubMed]
34. Sagi, D., & Tanne, D. (1994). Perceptual learning: learning to see. *Current opinion in neurobiology*, *4*(2), 195–199. [PubMed]
35. Yotsumoto Y, Chang LH, Watanabe T & Sasaki Y Interference and feature specificity in visual perceptual learning. *Vision Res* 49, 2611–2623 (2009). [PubMed]
36. Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. *Sleep*, *33*(5), 647–656. [PubMed]

37. Driver, H. S., Werth, E., Dijk, D.-J., & Borbély, A. A. (2008). The Menstrual Cycle Effects on Sleep. *Sleep Medicine Clinics*, *3*(1), 1–11. [ScienceDirect]
38. Driver, H. S., & Baker, F. C. (1998). Menstrual factors in sleep. *Sleep Medicine Reviews*, *2*(4), 213–229. [ScienceDirect]
39. Baker, F. C., & Driver, H. S. (2004). Self-reported sleep across the menstrual cycle in young, healthy women. *Journal of Psychosomatic Research*, *56*(2), 239–243. [ScienceDirect]
40. Hensch TK. Critical period plasticity in local cortical circuits. *Nature reviews Neuroscience.* 2005;6:877–888. [PubMed]
41. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. *Current biology: CB.* 2011;21:480–484. [PubMed]
42. Kim S, Stephenson MC, Morris PG, Jackson SR. tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: a 7 T magnetic resonance spectroscopy study. *NeuroImage.* 2014;99:237–243. [PubMed]
43. Nikolova S, Stark SM, Stark CE. 3T hippocampal glutamate-glutamine complex reflects verbal memory decline in aging. *Neurobiology of aging.* 2017;54:103–111. [PubMed]
44. Bang, J. W., Shibata, K., Frank, S. M., Walsh, E. G., Greenlee, M. W., Watanabe, T., & Sasaki, Y. (2018). Consolidation and reconsolidation share behavioral and neurochemical mechanisms. *Nature human behavior*, *2*(7), 507–513. [PubMed]
45. Tamaki, M., Wang, Z., Barnes-Diana, T., Guo, D., Berard, A. V., Walsh, E., Watanabe, T., & Sasaki, Y. (2020). Complementary contributions of non-REM and REM sleep to visual learning. *Nature neuroscience*, *23*(9), 1150–1156. [PubMed]
46. Hagemann, G., Ugur, T., Schleussner, E., Mentzel, H. J., Fitzek, C., Witte, O. W., & Gaser, C. (2011). Changes in brain size during the menstrual cycle. *PloS one*, *6*(2), e14655. [PubMed]
47. Mercadante, A. A., & Tadi, P. (2023). Neuroanatomy, Gray Matter. In *StatPearls*. StatPearls Publishing. [PubMed]
48. Driemeyer, J., Boyke, J., Gaser, C., Büchel, C., & May, A. (2008). Changes in gray matter induced by learning--revisited. *PloS one*, *3*(7), e2669. [PubMed]

49. Oh, C. M., Kim, H. Y., Na, H. K., Cho, K. H., & Chu, M. K. (2019). The Effect of Anxiety and Depression on Sleep Quality of Individuals With High Risk for Insomnia: A Population-Based Study. *Frontiers in neurology*, *10*, 849. [PubMed]
50. Nillni, Y. I., Toufexis, D. J., & Rohan, K. J. (2011). Anxiety sensitivity, the menstrual cycle, and panic disorder: a putative neuroendocrine and psychological interaction. *Clinical psychology review*, *31*(7), 1183–1191. [PubMed]
51. Handy, A. B., Greenfield, S. F., Yonkers, K. A., & Payne, L. A. (2022). Psychiatric Symptoms Across the Menstrual Cycle in Adult Women: A Comprehensive Review. *Harvard review of psychiatry*, *30*(2), 100–117. [PubMed]
52. Ghodasara SL, Davidson MA, Reich MS, et al. Assessing Student Mental Health at the Vanderbilt University School of Medicine. *Acad Med.* 2011;86 [PubMed]
53. Wilson, R. C., Takahashi, Y. K., Schoenbaum, G. & Niv, Y. Orbitofrontal cortex as a cognitive map of task space. *Neuron* 81, 267–278 (2014). [PubMed]
54. Mante, V., Sussillo, D., Shenoy, K. V. & Newsome, W. T. Context-dependent computation by recurrent dynamics in prefrontal cortex. *Nature* 503, 78–84 (2013). [PubMed]
55. Mack, M. L., Preston, A. R., & Love, B. C. (2020). Ventromedial prefrontal cortex compression during concept learning. *Nature communications*, *11*(1), 46 [PubMed]
56. Cao, N., Pi, Y., Qiu, F., Wang, Y., Xia, X., Liu, Y., & Zhang, J. (2022). Plasticity changes in dorsolateral prefrontal cortex associated with procedural sequence learning are hemisphere-specific. *NeuroImage*, *259*, 119406. [PubMed]
57. Pereira, S. I. R., & Lewis, P. A. (2020). Sleeping through brain excitation and inhibition. *Nature neuroscience*, *23*(9), 1037–1039. [PubMed]