Menstrual cycle-modulated intrinsic connectivity enhances olfactory performance during periovulatory period*

Yun-Ting Chao[1,2,3], Tzu-Yi Hong[1,3], Jen-Chuen Hsieh[1,3,4,5,6]

[1] Institute of Brain Science, National Yang Ming Chiao Tung University, Taipei, Taiwan

[2] Division of Rhinology, Department of Otorhinolaryngology-Head and Neck Surgery, Taipei Veterans General Hospital, Taipei, Taiwan

[3] Integrated Brain Research Unit, Division of Clinical Research, Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan

[4] Department of Biological Science and Technology, College of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan

[5] Brain Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan

[6] Center for Intelligent Drug Systems and Smart Bio-devices, National Yang Ming Chiao Tung University, Hsinchu Taiwan

Rhinology 61: 5, 441 - 448, 2023

https://doi.org/10.4193/Rhin22.472

***Received for publication:** December 9, 2022

Accepted: March 13, 2023

Abstract

Background: Olfactory capacity increases during the period of ovulation, perhaps as an adjunct to mate selection; however, researchers have yet to elucidate the neural underpinning of menstrual cycle-dependent variations in olfactory performance.

Methodology: A cohort of healthy volunteers (n = 88, grand cohort) underwent testing for gonadal hormone levels and resting-state functional magnetic resonance imaging with a focus on intrinsic functional connectivity (FC) in the olfactory network based on a priori seeds (piriform cortex and orbitofrontal cortex) during the periovulatory (POV) and menstrual (MEN) phases. A subcohort (n = 20, olfaction cohort) returned to the lab to undergo testing of olfactory performance during the POV and MEN phases of a subsequent menstrual cycle.

Results: Olfactory performance and FC were both stronger in the periovulatory phase than in the menstrual phase. Enhanced FC was observed in the network targeting the cerebellum in both the grand and olfaction cohorts, while enhanced FC was observed in the middle temporal gyrus, lingual gyrus, dorsal medial prefrontal cortex, and postcentral gyrus in the grand cohort. Periovulatory progesterone levels in the grand cohort were positively correlated with FC in the network targeting the insula and paracentral lobule.

Conclusions: Our analysis revealed that superior olfactory function in the periovulatory period is associated with enhanced intrinsic connectivity in the olfactory network. These findings can be appreciated in the context of evolutionary biology.

Key words: menstrual cycle, olfactory network, progesterone, periovulatory phase

Introduction

Women outperform men in all aspects of olfactory ability [(1)] in all age groups [(2)] and in all cultures [(3)]. Several hypotheses have been posited to explain this discrepancy. From a behavioral perspective, odors can be viewed as social signals [(4)]. Women tend to spend more time preparing food, which makes them more conscious of odors [(1)]. Researchers have also speculated that it is associated with the selection of sexual partners [(5)]. Women generally rate olfaction as the single sensory modality with the most pronounced effect on sexual responsiveness and mate choice, whereas men generally rate visual information as high or higher than olfactory information. Interestingly, preferences for body odor vary throughout the menstrual cycle. Shortly before or during ovulation, women tend to favor the scent of males who also present phenotypic markers indicative of genetic superiority, such as body symmetry [(6)] and dominant behavior

over other males [7]. Around the time of ovulation, women tend to show increased sensitivity [8-11] and hedonic preference [12] to odors of relevance to the choice of partner (e.g., androstenone or musky smells). Moreover, the sensitivity to a wider variety of odors (not limited to the male scents) appears to exhibit identical menstrual cycle-dependent variations [11, 13-16].

Olfactory changes throughout the menstrual cycle can be attributed to physiological fluctuations or altered neural processing [17]. Cyclic hormonal change is a major physiological factor that influences a wide spectrum of mental and behavioral activities, including cognition, emotion, and non-verbal behavior [18], and this can also be extrapolated to olfactory function. Early in life, the development of the olfactory system is related to the endocrine system (e.g., estradiol and testosterone) [19]. Later in life, circulating gonadal hormones play a critical role in maximizing odor discrimination capacity [20], promoting neurogenesis of the olfactory bulb (estradiol) [21], preventing oxidative stress in the olfactory system (estradiol) [22], and modulating the response evoked by odorants in olfactory receptor neurons (progesterone and estradiol) [23]. However, it remains unclear which hormones (e.g., progesterone, estradiol, or testosterone) regulate phasic olfactory changes or how this comes about. It also remains unclear whether this hormonal control is modulated by central neural processing.

Connectivity in the main intrinsic networks (default mode, executive control, and salience networks) has been shown to vary throughout the menstrual cycle [24-27]. Furthermore, the neural correlates underlying cognition, emotion, attention, coordination, contextual memory, and sensorimotor function are affected by gonadal hormones [28] and menstrual phases [25, 26, 29-31]. Neural networks related to sensory functions (e.g., visual, auditory, and olfactory) also fluctuate in response to hormonal changes [26, 27]. One study reported that alterations in the olfactory network (ON) were associated with oral contraceptives; however, no significant menstrual cycle-dependent effect was observed [26].

In the current study, we investigated dynamic changes in the ON throughout the menstrual cycle based on functional connectivity (FC) analysis of resting-state functional magnetic resonance imaging (rs-fMRI). We hypothesized that the sense of smell and intrinsic connectivity in the ON are both stronger around the time of ovulation. We also investigated the effects of changes in serum gonadal hormone levels on neural network plasticity.

Materials and methods

Subjects

The subjects enrolled in this study were healthy control participants recruited from our *Neuroimaging Program of Primary Dysmenorrhea*, a portion of which has previously been published [32]. Participants in that program were women with a regular menstrual cycle of 27-32 days. None of the subjects enrolled in the control group had dysmenorrhea. The program involved a battery of assessments, including gonadal hormone levels and multimodal neuroimaging studies (rs-fMRI and structural MRI) during the periovulatory (POV) and menstrual (MEN) phases, respectively. None of the participants were pregnant or using oral contraceptives at the time of the study. Participants with physical or neurological disorders and/or MRI contraindications were excluded. We also excluded subjects who had head movements greater than 2 mm or 2 degrees during MRI preprocessing.
The final cohort consisted of 88 female subjects (termed grand cohort, mean age: 23.8 ± 2.7 years old). A subset of participants (termed the olfaction cohort, n = 20; mean age 24.8 ± 2.7 years old) from the grand cohort was randomly recruited to return to the lab and undertake an evaluation of olfactory function. Note that we originally intended to recruit more participants for the olfaction cohort; however, our attempts were thwarted by the COVID-19 pandemic. All participants were right-handed, as confirmed by the Edinburgh Inventory [33]. The current study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee of Taipei Veterans General Hospital with informed consent provided by each individual.

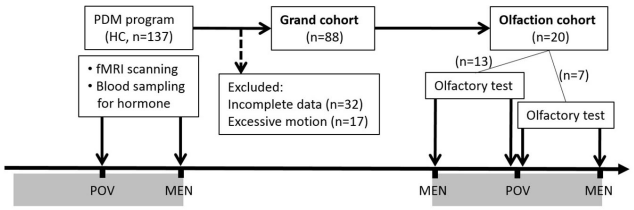

Figure 1. Schematic diagram of the study design. The study recruited healthy control participants (HC) from the Neuroimaging Program of Primary Dysmenorrhea (PDM). Participants underwent testing for gonadal hormone levels and resting-state fMRI during the periovulatory phase (POV) and menstrual phase (MEN). After excluding 49 subjects, the remaining 88 participants (grand cohort) were analyzed. Additionally, a subset of the grand cohort (olfaction cohort, n=20) underwent olfactory tests starting either in the MEN (n=13) or POV (n=7) phase. The gray column represents one menstrual cycle.

Experimental design

Figure 1 illustrates the experimental flowchart of the current study. Experiment schedules were organized in accordance with individual menstrual cycles, where the first day of menstruation served as day 1. MRI images (T1 and rs-fMRI images) were acquired once during the MEN phase (days 1-3 of the menstrual cycle) and once during the POV phase (days 12-16 of the menstrual cycle). At the same times, blood samples were taken for gonadal hormone assays. Participants in the olfaction cohort

additionally underwent an assessment of olfactory performance during the MEN and POV phases. Due to technical difficulties, the olfactory tests were not conducted during the same cycles as the MRI scanning and hormone measurements were obtained. The order of phases during which the participants underwent olfactory test (MEN first or POV first) was randomized.

Serum gonadal hormone levels

Blood samples were drawn during the MEN and POV phases. Serum was extracted and stored for batch analysis using commercialized assays (UniCel DxC 800 Synchron Clinical Systems, Beckman Coulter, Inc., Brea, CA, USA). Total serum concentrations were tested using a chemiluminescence immunoassay technique for estradiol and progesterone and a radioimmunoassay technique for testosterone. Supplementary Table 1 presents the phase difference of each hormone.

Olfactory performance

Olfactory performance was evaluated using the Sniffin' Sticks test (Burghart Instruments, Wedel, Germany), which is a set of felt tip pens used to present odors. The comprehensive exam comprises three subtests: odor detection threshold (T), odor discrimination (D), and odor identification (I) [34]. The olfactory threshold for n-butanol was assessed using the adaptive staircase procedure with a forced choice technique of three alternatives. The odor discrimination task used 16 triplets of sticks, two of which contained the same odor, and the third contained a different odor. The odor probes were randomly presented to the participants, who were supposed to identify the divergent one. Odor identification involved 16 common odors, which were identified from a list of descriptors (four for each odor). The scores of the three subtests were summed into a composite TDI score. Nasal conditions and emotional states have been shown to influence olfactory performance; therefore, we also required that participants complete the Sino-Nasal Outcome Test (SNOT-22), Beck Depression Inventory (BDI-II), and Beck Anxiety Inventory (BAI) questionnaires. The paired t-test was performed to evaluate changes between phases.

MRI acquisition

The images were obtained at National Yang Ming Chiao Tung Universityusing a 3.0 Tesla MR scanner (Magnetom Trio Tim, Siemens, Erlangen, Germany) with a 12-channel head coil. High-resolution T1 weighted 3-dimensional structural images were acquired using a magnetization prepared rapid acquired gradient echo sequence (MPRAGE; [TR]/[TE] = 2530 ms/3.03 ms, flip angle = 70°, field of view = 224 × 256 × 192 mm^3, in-plane matrix size = 224 × 256 × 192, in-plane resolution = 1 mm). The rs-fMRI images were echo-planar imaging (EPI) obtained using a weighted gradient echo sequence ([TR]/[TE] = 2500 ms/30 ms, flip angle = 90°, field-of-view = 220 × 220 mm^2, in-plane matrix size = 64 × 64 × 40; in-plane resolution = 3.4 mm, and 200 volumes). Head cushions and ear plugs were provided to reduce head motion and noise, respectively. Participants remained awake throughout the scanning session in a resting state (eyes open and head still without thinking about anything).

Pre-processing of fMRI data

The images were pre-processed using the DPARSF 4.5 Toolbox (State Key Laboratory for Cognitive Neuroscience and Learning, Beijing Normal University, China) [35] in conjunction with Statistical Parametrical Mapping 12 (SPM12, Wellcome Trust Center for Neuroimaging, London, http://www.fil.ion.ucl.ac.uk/spm) within a MATLAB framework (MATLAB R2020b, The MathsWorks Inc., Natick, MA, USA). All functional images were subjected to slice timing and realignment. Subjects who presented head motion of any volume greater than 2 mm or 2 degrees were excluded from further processing. The Friston 24-parameter model was used to regress the head motion effect. Other sources of noise were regressed by removing white matter and CSF signals. We then performed the non-linear registration of individual EPI images on an EPI template. The images were resampled to an isotropic 2 × 2 × 2 mm^3 voxel size during the normalization step and spatially smoothed using a 3D Gaussian kernel of 6 mm full width at half maximum. Note that we opted not to perform global signal regression, as this is a controversial practice in FC studies.

Seed-based FC maps and statistical analysis

Local neural networks are generally organized into cortical representations induced by stimulus or task; therefore, we defined bilateral pyriform cortices (PC) and orbital frontal cortices (OFC) with a radius of 5 mm as anatomical ON seed areas based on a meta-analysis of fMRI studies of olfactory activation [36]. PC-seeded ON was centered at MNI coordinates (-22, 0, -14) and (22, 2, -12), while OFC-seeded ON was centered at (-24, 30, -10) and (28, 34, -12). After the extraction of the mean time series activity from each seed region, seed-based FC maps were generated for group analysis. Each FC map at the individual level was then converted into a z map using Fisher's r-to-z transformation for second-level group analysis.

Statistical analysis

Changes in behavior-related metrics and hormone levels between the POV and MEN phases were assessed using a paired t-test implemented in SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, NY, USA). The study used a repeated measures analysis of variance (rmANOVA) to examine the impact of the order of measurement (treated as the between-subject variable) on changes in composite TDI scores, with "phase" as the within-subject variable. The level of significance was set at $p < 0.05$. A within-subject between-phase comparison of olfac-

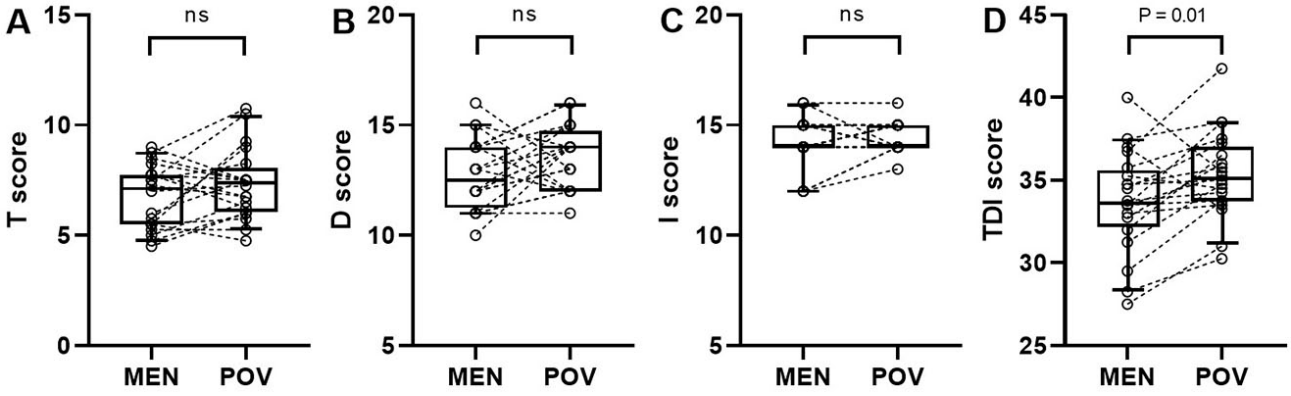

Figure 2. Olfactory performance during the menstrual (MEN) and periovulatory (POV) phases. The box plot revealed no significant difference between phases for (A) threshold score (T), (B) discrimination score (D), and (C) identification score (I). (D) A significant difference was observed in the composite TDI score (paired t = 2.73, p = 0.01). Each pair of circles connected by a dashed line represented individual changes in olfactory measures. The median was represented by the line within the box, while the lower and upper boundaries of the box represented the 25th and 75th percentiles, respectively. The whiskers below and above the box represented the 10th and 90th percentiles, respectively. "ns" was used to indicate non-significance.

tory seed-based FC maps was performed using a paired t-test implemented in SPM12. Serum estradiol, progesterone, and testosterone data were then correlated with the FC map in the POV and MEN phases using a one-sample t-test. The coordinates that survived thresholding of voxel level $p < 0.001$ and further family-wise error (FWE)-corrected cluster level $p < 0.05$ were deemed significant.

Results

Superior olfactory performance during the POV phase

We first examined dynamic changes in olfactory performance throughout the menstrual cycle. Figure 1 shows that the participants were randomly assigned to begin in either the MEN phase (n=13) or the POV phase (n=7). Note that this resulted in significantly higher composite TDI scores in the POV phase (two-tailed paired t = 2.73, P = 0.01, Figure 2). No significant phase differences were observed in terms of olfactory subtests, sinonasal symptoms, depression levels, or anxiety scores (Supplementary Table 2). The rmANOVA analysis showed that there was a significant effect of "phase" on TDI scores (F [1, 18] = 5.60, P = 0.03). However, the effect of "order of measurement" (F [1, 18] = 0.50, P = 0.49) and the interaction of "phase" x "order of measurement" (F [1, 18] = 0.47 P = 0.50) were not significant. These results suggest that the changes observed in TDI scores were not due to retest bias. To sum up, among subjects in the olfaction cohort, olfactory performance during the POV phase was better than that during the MEN phase.

Involvement of the olfactomotor system during the POV phase

We then examined dynamic changes in the neural organization of the ON in the olfaction cohort throughout the menstrual cycle. In whole brain rs-fMRI analysis, the OFC-seeded ON presented elevated FC targeting the cerebellum area XIII-IX during the POV phase (Figure 3A and Supplementary Table 3). Note that enhanced FC of the ON was not observed during the MEN phase. Taken together, these results indicate elevated intrinsic connectivity in the ON involving the cerebellum (generally regarded as an element of the olfactomotor system [37]) during the POV phase.

Generalized strengthening of ON connectivity during the POV phase

Next, we sought to verify whether the observed menstrual cycle-dependent engagement of olfactomotor systems was generalizable to the overall grand cohort (as a plausible representation of the overall population) by comparing the respective ONs during the POV and MEN phases. The grand cohort was subjected to the same methods used in the olfactory cohort. During the POV phase, PC-seeded ON was shown to engage not only the olfactomotor system (cerebellum area VI) but also the middle temporal gyrus, lingual gyrus, dorsal medial prefrontal cortex, and postcentral gyrus (Figure 3B and Supplementary Table 4), which are all known parts of the ON [38, 39]. In contrast, the ON did not present increased intrinsic connectivity during the MEN phase. Taken together, these behavioral and brain imaging findings strongly suggest that superior olfactory performance during the POV phase can be attributed to enhanced intrinsic connectivity in the ON.

Correlation between ON connectivity and progesterone levels during the POV phase

Our final objective was to elucidate the role of gonadal hormone levels in modulating cyclic changes in the ON. Progesterone le-

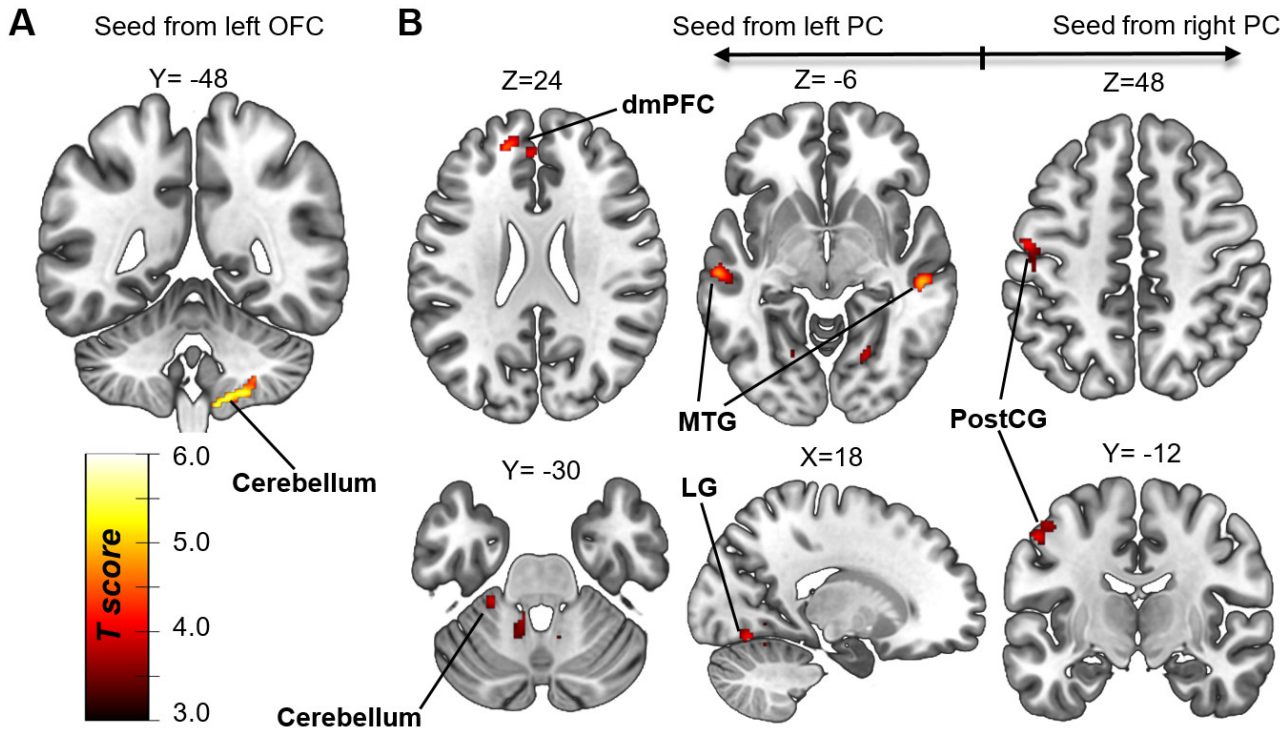

Figure 3. Representations of changes in the olfactory network (POV > MEN phase) in (A) the olfaction cohort (n = 20) and in (B) the grand cohort (n = 88). MTG, middle temporal gyrus; LG, lingual gyrus; dmPFC, dorsal medial prefrontal cortex; PostCG, postcentral gyrus.

vels in the POV phase were positively correlated with PC-seeded ON connectivity to the paracentral lobule and OFC-seeded ON connectivity to the paracentral lobule and insula (Figure 4 and Supplementary Table 5). No significant correlation was observed for estradiol or testosterone during the POV phase or for the three hormones during the MEN phase. These findings suggested that progesterone is the critical hormone mediating ON neuroplasticity, particularly during the POV phase.

Discussion

The present study investigated menstrual cycle-dependent changes in intrinsic connectivity in the ON. Our results indicate that enhanced olfactory performance in the POV phase can be attributed to enhanced connectivity in the ON. Olfactory performance favoring the POV phase was in line with previous studies [8-11, 13-15]. One previous study using the same test battery (Sniffin' Sticks test) reported superior threshold levels during the follicular phase, superior identification in the luteal phase, and no variation in discrimination [15]. In the current study, we did not observe significant variations in subtest scores. This discrepancy can perhaps be attributed to the study design. Unlike this study, the previous study was not a within-subject longitudinal design, and the phases of interest were different. Our results provide evidence that the overall olfactory function of women improves during the period of ovulation.

Despite not meeting the minimally clinical important difference (MCID) of 5.5 points for TDI scores [40], our study still found significant phasic differences in olfactory function throughout the menstrual cycle. The proposed MCID was based on patients with hyposmia / anosmia who had substantial olfactory dysfunction. However, psychophysical tests and more sensitive neuroimaging techniques can detect subtle changes in olfactory functionality in normosmic individuals. Moreover, the randomized order of measurement was used to control for retest bias, minimizing the possibility that the test repetition (with weeks in between) contributed to the observed changes in olfactory ability.

Analysis of rs-fMRI revealed menstrual cycle-dependent alterations in intrinsic connectivity. We demonstrated that during the POV phase, PC-seeded ON in the grand cohort significantly engaged the cerebellum area VI, middle temporal gyrus, lingual gyrus, dorsal medial prefrontal cortex, and postcentral gyrus. These regions of the brain are considered functional hubs for olfactory processing pathways originating in the primary olfactory cortex [38, 39]. In particular, cerebellar area VI is frequently activated during odor exposure [41], and its gray matter volume is positively correlated with olfactory performance [42]. The olfaction cohort also presented stronger OFC-seeded FC in cerebellum area XIII-IX, which serves as an important hub for intrinsic cerebellar connectivity related to olfaction [41]. These findings confirm the

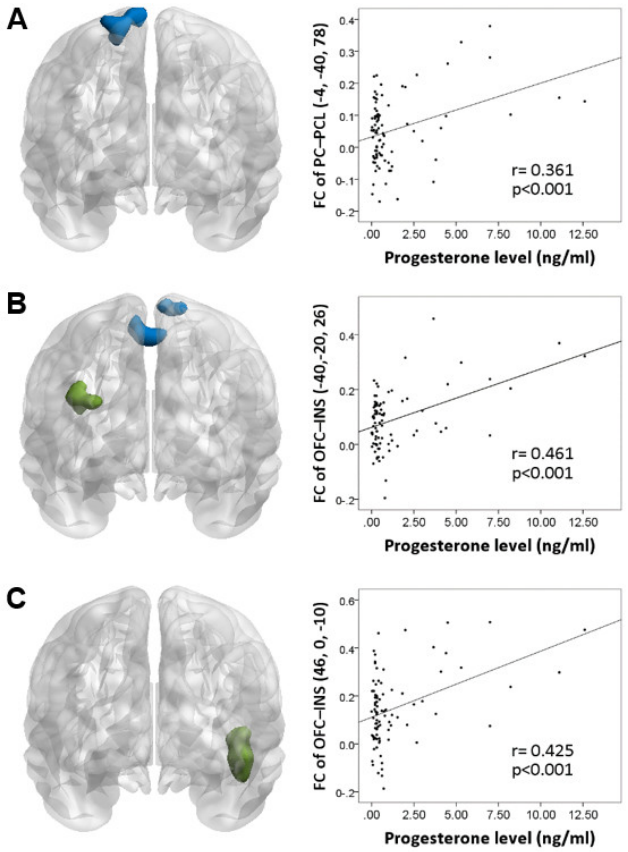

Figure 4. Clusters in olfactory networks correlating with serum progesterone levels during the POV phase in the grand cohort (n = 88). (A) Right PC-seeded olfactory network; (B) left OFC-seeded olfactory network; (C) right OFC-seeded olfactory network. Clusters in blue, paracentral lobule; clusters in green, insula.

existence of ON-cerebellum coupling and highlight the role of the cerebellum in the olfactory processing system during the period of ovulation. The cerebellum has also been linked to the olfactomotor system, wherein it regulates intranasal airflow in response to chemosensory stimulation [37]. We posit that superior olfactory performance during ovulation is fine-tuned by the olfactomotor system. Coupling of the olfactory and olfactomotor systems during the POV phase may explain the variability in olfactory function throughout the menstrual cycle.

The grand cohort (n = 88) was four times the size of the olfaction cohort (n = 20), which means that the analytic results from the grand cohort (e.g., cerebellum area VI) may yield the most stable and robust neural dynamics. It is far more likely that the results from the smaller olfaction cohort (e.g., cerebellum area XIII-IX) were influenced by the diversity of individual ONs, which can be highly vulnerable to the process of averaging [32].

Women of reproductive age can conceive only during the ovulatory phase, which tends to enhance their sensitivity in selecting a suitable sexual partner. Suitability can often be viewed as traits imbuing potential genetic benefits for the offspring. Women in their fertile period tend to be attracted to masculine men with symmetrical faces [43], a masculine voice [44], and displays of dominance [45]. In blind tests as well, researchers have observed a menstrual cycle-dependent preference for the scent of males with symmetrical bodies [6] or dominant behavior [7]. It has been proposed that this odor-signaling communication is regulated by the major histocompatibility complex (MHC), a set of antigen-presenting molecules involved in human immunity [46]. Women's MHC may guide their attention towards dissimilar genotypes [47], particularly those with higher chances of producing infection-resistant offspring through heterozygosity [48]. In one study, participants exhibited faster and larger evoked potentials in response to the body odors of individuals with a similar MHC genotype [49]. It is likely that this processing confers an advantage in the avoidance of MHC-similar partners. In summary, preferences in MHC-related body odor may explain why female olfactory performance increases during the ovulatory period. The current neuroimaging results add to our understanding of the mechanism underlying olfactory changes throughout the menstrual cycle.

Our findings also suggest that progesterone plays an important role in modulating the ON. Endogenous progesterone is cyclically elevated in the luteal (non-fertile) phase and may inactivate male pheromone-responsive neurons [50]. Odorant-evoked responses in olfactory receptor neurons [23] and the neural networks associated with memory and somatosensory systems [28, 29] are also modulated by circulating progesterone. In the current study, progesterone levels were positively correlated with FC from the core ON seeds to the insula and paracentral lobule (Figure 4). These target areas are not identical to the areas in Figure 3; however, they are also important neural substrates of the ON. The insula and paracentral lobule serve as cardinal hubs in the somatosensory network, which is responsible for intranasal trigeminal function and chemosensory integration [39, 51]. This suggests that progesterone plays a role in promoting the functional convergence of olfaction with other sensory perception during the POV phase. It should be borne in mind that hormone measurements and olfactory testing were not conducted during the same menstrual cycle. Future research involving simultaneous assessments will be required to confirm the relationship between progesterone levels and olfactory function during the POV phase.

Conclusion

Women of reproductive age exhibit menstrual cycle-dependent plasticity in the ON manifesting as fluctuations in olfactory performance. During the ovulatory period, an improved sense of smell (underpinned neurologically by enhanced intrinsic con-

nectivity of the ON) may confer an evolutionary advantage in terms of mate choice and offspring viability.

Acknowledgements

We would like to thank Mr. Wei-Chi Lee for his technical assistance with the fMRI; Mr. Wei-Chi Lee, Ms. Tzu-Ling Tseng, and Prof. Li-Fen Chen for their intellectual contribution to the Neuroimaging Program of Primary Dysmenorrhea; and Prof. Thomas Hummel (TU Dresden, Germany) for his valuable suggestions. This work was supported by the Ministry of Science and Technology (MOST 108-2314-B-075-012, MOST 110 -2927-I-010 -504, MOST 111-2628-B-075 -010 to Y.-T.C. ; and NSC 100-2314-B-010-006-MY3, NSC 100-2629-B-010-001, NSC 101-2629-B-010-001, NSC 102-2629-B-010-001, MOST 106-2629-B-010-001-MY3, MOST 108-2314-B-010-026, MOST 109-2314-B-010-037-MY2 to J.-C.H.), Taipei Veterans General Hospital (V108B-025 to Y.-T.C.; and V100D-001, V108C-122, V109C-142, V110C-035 to J.-C.H.), Taipei Veterans General Hospital – National Taiwan University Hospital Joint Research Program (VN103-05, VN104-03, VN105-03 to J.-C.H.), Yin Shu-Tien Foundation – Taipei Veterans General Hospital – National Yang-Ming University Excellent Physician-Scientist Cultivation Program (111-V-B-002, 108-V-B-021 to Y.-T.C.), and Brain Research Center, National Yang Ming Chiao Tung University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

Authorship contribution

Y.-T.C. and J.-C.H. contributed to the conception and design of the study. Y.-T.C. and T.-Y.H. contributed to data acquisition and image processing. Y.-T.C. analyzed the data. Y.-T.C. and J.-C.H. interpreted the data and drafted the manuscript with input from T.-Y.H. J.-C.H. approved the final submission.

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Sorokowski P, Karwowski M, Misiak M, et al. Sex differences in human olfaction: a meta-analysis. Front Psychol. 2019; 10: 242.
2. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. Science. 1984; 226: 1441-3.
3. Doty RL, Applebaum S, Zusho H, Settle RG. Sex differences in odor identification ability: a cross-cultural analysis. Neuropsychologia. 1985; 23: 667-72.
4. Croy I, Springborn M, Lötsch J, Johnston ANB, Hummel T. Agreeable smellers and sensitive neurotics – correlations among personality traits and sensory thresholds. PLOS ONE. 2011; 6: e18701.
5. Herz RS, Inzlicht M. Sex differences in response to physical and social factors involved in human mate selection: The importance of smell for women. Evolution Hum Behav. 2002; 23: 359-64.
6. Gangestad SW, Thornhill R. Menstrual cycle variation in women's preferences for the scent of symmetrical men. Proc Biol Sci. 1998; 265: 927-33.
7. Havlicek J, Roberts SC, Flegr J. Women's preference for dominant male odour: effects of menstrual cycle and relationship status. Biol Lett. 2005; 1: 256-9.
8. Mair RG, Bouffard JA, Engen T, Morton TH. Olfactory sensitivity during the menstrual cycle. Sens Processes. 1978; 2: 90-8.
9. Vierling JS, Rock J. Variations in olfactory sensitivity to exaltolide during the menstrual cycle. J Appl Physiol. 1967; 22: 311-5.
10. Renfro KJ, Hoffmann H. The relationship between oral contraceptive use and sensitivity to olfactory stimuli. Horm Behav. 2013; 63: 491-6.
11. Caruso S, Grillo C, Agnello C, Maiolino L, Intelisano G, Serra A. A prospective study evidencing rhinomanometric and olfactometric outcomes in women taking oral contraceptives. Hum Reprod. 2001; 16: 2288-94.
12. Hummel T, Gollisch R, Wildt G, Kobal G. Changes in olfactory perception during the menstrual cycle. Experientia. 1991; 47: 712-5.
13. Navarrete-Palacios E, Hudson R, Reyes-Guerrero G, Guevara-Guzmán R. Lower olfactory threshold during the ovulatory phase of the menstrual cycle. Biol Psychol. 2003; 63: 269-79.
14. Doty RL, Snyder PJ, Huggins GR, Lowry LD. Endocrine, cardiovascular, and psychological correlated of olfactory sensitivity changes during the human menstrual cycle. J Comp Physiol Psychol. 1981; 95: 45-60.
15. Derntl B, Schopf V, Kollndorfer K, Lanzenberger R. Menstrual cycle phase and duration of oral contraception intake affect olfactory perception. Chem Senses. 2013; 38: 67-75.
16. Pause BM, Sojka B, Krauel K, Fehm-Wolfsdorf G, Ferstl R. Olfactory information processing during the course of the menstrual cycle. Biol Psychol. 1996; 44: 31-54.
17. Martinec Novakova L, Havlicek J, Roberts S. Olfactory processing and odor specificity: A meta-analysis of menstrual cycle variation in olfactory sensitivity. Anthropol Rev. 2014; 77: 331–45.
18. van Wingen GA, Ossewaarde L, Backstrom T, Hermans EJ, Fernandez G. Gonadal hormone regulation of the emotion circuitry in humans. Neuroscience. 2011; 191: 38-45.
19. Doty RL, Cameron EL. Sex differences and reproductive hormone influences on human odor perception. Physiol Behav. 2009; 97: 213-28.
20. Kunkhyen T, Perez E, Bass M, Coyne A, Baum MJ, Cherry JA. Gonadal hormones, but not sex, affect the acquisition and maintenance of a Go/No-Go odor discrimination task in mice. Horm Behav. 2018; 100: 12-9.
21. Brus M, Trouillet AC, Hellier V, Bakker J. Estradiol-induced neurogenesis in the female accessory olfactory bulb is required for the learning of the male odor. J Neurochem. 2016; 138: 457-68.
22. Bernal-Mondragón C, Rivas-Arancibia S, Kendrick KM, Guevara-Guzmán R. Estradiol prevents olfactory dysfunction induced by A-β 25-35 injection in hippocampus. BMC Neurosci. 2013; 14: 104.
23. Kanageswaran N, Nagel M, Scholz P, Mohrhardt J, Gisselmann G, Hatt H. Modulatory effects of sex steroids progesterone and estradiol on odorant evoked responses in olfactory receptor neurons. PLoS One. 2016; 11: e0159640.
24. Petersen N, Kilpatrick LA, Goharzad A, Cahill L. Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. Neuroimage. 2014; 90: 24-32.
25. Engman J, Sundström Poromaa I, Moby L, Wikström J, Fredrikson M, Gingnell M. Hormonal cycle and contraceptive effects on amygdala and salience resting-state networks in women with previous affective side effects on the pill. Neuropsychopharmacol. 2018; 43: 555-63.
26. Pletzer B, Crone JS, Kronbichler M, Kerschbaum H. Menstrual cycle and hormo-

nal contraceptive-dependent changes in intrinsic connectivity of resting-state brain networks correspond to behavioral changes due to hormonal status. Brain Connect. 2016; 6: 572-85.
27. Weis S, Hodgetts S, Hausmann M. Sex differences and menstrual cycle effects in cognitive and sensory resting state networks. Brain Cogn. 2019; 131: 66-73.
28. Syan SK, Minuzzi L, Costescu D, et al. Influence of endogenous estradiol, progesterone, allopregnanolone, and dehydroepiandrosterone sulfate on brain resting state functional connectivity across the menstrual cycle. Fertil Steril. 2017; 107.
29. Arélin K, Mueller K, Barth C, et al. Progesterone mediates brain functional connectivity changes during the menstrual cycle-a pilot resting state MRI study. Front Neurosci. 2015; 9: 44.
30. Hidalgo-Lopez E, Mueller K, Harris T, Aichhorn M, Sacher J, Pletzer B. Human menstrual cycle variation in subcortical functional brain connectivity: a multimodal analysis approach. Brain Struct Funct. 2020; 225: 591-605.
31. Thimm M, Weis S, Hausmann M, Sturm W. Menstrual cycle effects on selective attention and its underlying cortical networks. Neurosci. 2014; 258: 307-17.
32. Chao YT, Hong TY, Yang CJ, Hsieh JC. Variant brain-derived neurotrophic factor val66met polymorphism engages memory-associated systems to augment olfaction. Sci Rep. 2022; 12: 20007.
33. Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia. 1971; 9: 97-113.
34. Oleszkiewicz A, Schriever VA, Croy I, Hähner A, Hummel T. Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. Eur Arch Otorhinolaryngol. 2019; 276: 719-28.
35. Chao-Gan Y, Yu-Feng Z. DPARSF: A MATLAB Toolbox for "Pipeline" Data Analysis of Resting-State fMRI. Front Syst Neurosci. 2010; 4: 13.
36. Seubert J, Freiherr J, Djordjevic J, Lundstrom JN. Statistical localization of human olfactory cortex. Neuroimage. 2013; 66: 333-42.
37. Mainland J, Sobel N. The sniff is part of the olfactory percept. Chem Senses. 2006; 31: 181-96.
38. Zhou G, Lane G, Cooper SL, Kahnt T, Zelano C. Characterizing functional pathways of the human olfactory system. Elife. 2019; 8.
39. Tobia MJ, Yang QX, Karunanayaka P. Intrinsic intranasal chemosensory brain networks shown by resting-state functional MRI. Neuroreport. 2016; 27: 527-31.
40. Gudziol V, Lötsch J, Hähner A, Zahnert T, Hummel T. Clinical significance of results from olfactory testing. Laryngoscope. 2006; 116: 1858-63.
41. Zhang ZH, Liu X, Jing B, et al. Cerebellar involvement in olfaction: An fMRI Study. J Neuroimaging. 2021; 31: 517-23.
42. Wabnegger A, Schienle A. Cerebellar gray matter and olfactory performance. Chem Senses. 2019; 44: 507-10.
43. Little AC, Jones BC. Variation in facial masculinity and symmetry preferences across the menstrual cycle is moderated by relationship context. Psychoneuroendocrinol. 2012; 37: 999-1008.
44. Pisanski K, Hahn AC, Fisher CI, DeBruine LM, Feinberg DR, Jones BC. Changes in salivary estradiol predict changes in women's preferences for vocal masculinity. Horm Behav. 2014; 66: 493-7.
45. Gangestad SW, Simpson JA, Cousins AJ, Garver-Apgar CE, Christensen PN. Women's preferences for male behavioral displays change across the menstrual cycle. Psychol Sci. 2004; 15: 203-7.
46. Milinski M, Croy I, Hummel T, Boehm T. Major histocompatibility complex peptide ligands as olfactory cues in human body odour assessment. Proc Biol Sci. 2013; 280: 20122889.
47. Havlicek J, Roberts SC. MHC-correlated mate choice in humans: a review. Psychoneuroendocrinol. 2009; 34: 497-512.
48. Penn DJ. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology. 2002; 108: 1-21.
49. Pause BM, Krauel K, Schrader C, et al. The human brain is a detector of chemosensorily transmitted HLA-class I-similarity in same- and opposite-sex relations. Proc Biol Sci. 2006; 273: 471-8.
50. Dey S, Chamero P, Pru JK, et al. Cyclic regulation of sensory perception by a female hormone alters behavior. Cell. 2015; 161: 1334-44.
51. Kollndorfer K, Kowalczyk K, Frasnelli J, et al. Same same but different. Different trigeminal chemoreceptors share the same central pathway. PLoS One. 2015; 10: e0121091.

Jen-Chuen Hsieh, MD, PhD
Department of Biological Science and Technology
National Yang Ming Chiao Tung University
BioICT Building, Room 318
No. 75, Po-Ai Street
Hsinchu 300
Taiwan

Tel: +886-3-5712121#56990
E-mail: jchsiehibru@nycu.edu.tw

This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Hormone levels as a function of menstrual phase in the grand cohort (n=88).

Hormone	POV	MEN	Paired-t	p
Estradiol (pg/ml)	130.9 ±112.4	34.4 ±18.6	8.13	<0.001
Progesterone (ng/ml)	1.3 ±2.3	0.4 ±0.2	3.67	<0.001
Testosterone (ng/ml)	0.4 ±0.2	0.3 ±0.2	4.75	<0.001

POV, periovulatory phase; MEN, menstrual phase.

Supplementary Table 2. Behavioral factors in olfaction cohort (n=20).

Behavioral measurement (mean ± SD)	POV	MEN	Paired-t	p
Detection threshold	7.3 ±1.6	6.7 ±1.4	1.66	0.11
Odor discrimination	13.6 ±1.4	12.8 ±1.6	1.85	0.08
Odor identification	14.5 ±0.7	14.3 ±1.2	0.89	0.39
Composite TDI score	35.3 ±2.6	33.7 ±3.1	2.73	0.01*
Sino-nasal Outcome Test	9.9 ±11.6	9.8 ±8.9	0.07	0.95
Beck Depression Inventory	4.7 ±5.3	4.4 ±4.3	0.37	0.71
Beck Anxiety Inventory	3.2 ±5.5	2.5 ±2.7	0.75	0.47

POV, periovulatory phase; MEN, menstrual phase; SD, standard deviation; TDI, threshold + discrimination + identification. *p < 0.05

Supplementary Table 3. Altered intrinsic connectivity in olfactory network during POV phase compared with that during MEN phase in the olfaction cohort (n=20).

POV > MEN	MNI			Peak level		Cluster level		
Seeds	x	y	z	T	$p_{uncorr.}$	k	$p_{FWE-corr.}$	BA	Anatomic label
OFC (L) (-24, 30, -10)	10	-46	-60	5.82	<0.001	235	0.004	—	Cerebellum IX (R)
	22	-48	-54	5.55	<0.001				Cerebellum VIII (R)
	28	-48	-48	4.76	<0.001				Cerebellum VIII (R)

OFC, orbital frontal cortex; uncorr., uncorrected; FWE-corr., family-wise error-corrected; BA, Brodmann area; L, left; R, right.

Supplementary Table 4. Altered intrinsic connectivity in olfactory network during POV phase compared with that during MEN phase in the grand cohort (n=88).

POV > MEN	MNI			Peak level		Cluster level			
Seeds	x	y	z	T	$p_{uncorr.}$	k	$p_{FWE-corr.}$	BA	Anatomic label
PC (L) (-22, 0, -14)	54	-26	-4	5.09	<0.001	320	0.004	21	MTG(R)
	-60	-22	-6	4.90	<0.001	209	0.030	21	MTG (L)
	-16	48	24	4.64	<0.001	270	0.010	9	dmPFC (L)
	18	-70	-12	4.14	<0.001	245	0.015	19	LG (R)
	-26	-36	-30	4.01	<0.001	398	0.001	—	Cerebellum VI (L)
PC (R) (22, 2, -12)	-52	-10	48	4.24	<0.001	344	0.002	4	PostCG (L)
OFC (R) (28, 34, -12)	20	-12	58	4.27	<0.001	146	0.097	6	SMA (R)

PC, piriform cortex; OFC, orbital frontal cortex; uncorr., uncorrected; FWE-corr., family-wise error-corrected; BA, Brodmann area; MTG, middle temporal gyrus; dmPFC, dorsal medial prefrontal cortex; LG, lingual gyrus; PostCG, postcentral gyrus; SMA, supplementary motor area; L, left; R, right.

Supplementary Table 5. Altered intrinsic connectivity in olfactory networks correlating with serum progesterone levels during POV phase in the grand cohort (n=88)

	MNI			Peak level		Cluster level			
Seeds	x	y	z	T	$p_{uncorr.}$	k	$p_{FWE-corr.}$	BA	Anatomic label
PC (L) (-22, 0, -14)	-4	-40	78	3.96	<0.001	264	0.022	5	paracentral lobule
OFC (L) (-24, 30, -10)	-40	-20	26	4.67	<0.001	228	0.028	13	insula
	0	-30	56	4.12	<0.001	263	0.015	6	paracentral lobule
OFC (R) (28, 34, -12)	46	0	-10	4.49	<0.001	324	0.008	13	insula

PC, piriform cortex; OFC, orbital frontal cortex; uncorr., uncorrected; FWE-corr., family-wise error-corrected; BA, Brodmann area; L, left; R, right.