Variations of olfactory function with circadian timing and chronotype*

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Abstract

Background: Cumulative animal studies have suggested that olfaction can be regulated by circadian clock. However, human studies on the topic are relatively limited. The present study thus aimed to investigate diurnal variation in olfaction in healthy adults while examining potential modulating factors.

Methods: We conducted four rounds of testing on 56 healthy adults (32 women) aged 31 ± 12 years, throughout a single day, during morning (8:00-10:00 h), noon (12:00-14:00 h), afternoon (16:00-18:00 h), and evening (20:00-22:00 h). At the first appointment, participants completed full olfactory function testing using the Sniffin' Sticks, questionnaires on medical history, nasal symptoms, sleep quality, and chronotype, and were assessed for blood pressure, heart rate, peak nasal inspiratory flow (PNIF), attention level, and rated their smell ability, nasal patency, wakefulness, and concentration level using visual analog scale (VAS) ratings. Subsequent appointments measured olfactory threshold, attentional level, PNIF, blood pressure, heart rate and VAS ratings repeatedly.

Results: Olfactory threshold (OT) scores varied significantly between different times of the day, with the highest score in the evening and the lowest in the morning. Similar differences were also observed in PNIF, with the highest value in the evening and the lowest in the morning. However, there were no significant correlations between OT score and PNIF across all four tests, as well as between differences in [OT evening – OT morning] and [PNIF evening – PNIF morning]. Furthermore, a generalized linear mixed model indicated that the testing time of the morning, evening chronotype, self-reported body mass index (BMI), rated smell ability, and rated nasal patency significantly predicted the Sniffin' Sticks OT score.

Conclusions: Olfactory function fluctuates throughout the waking hours of the day, with the highest olfactory sensitivity observed in the evening and the lowest in the morning. This pattern is also seen in nasal patency. However, it appears that the circadian changes of nasal airflow may not significantly depend on the circadian changes of the olfactory sensitivity. In addition, chronotype and BMI may regulate such olfactory-circadian variation. These findings provide important insights for future research on the accurate diagnosis and treatment of olfactory dysfunction.

Key words: Circadian clock, chronotype, olfactory threshold, nasal airflow, PNIF

Introduction

The circadian clock has been observed in diverse organisms to integrate external environmental changes and internal physiological functions [1]. It plays a role in synchronizing biological processes, including body temperature, hunger, sleep, gene transcription, and sensory perceptions [2]. This endows the host with temporal precision and enhances adaptation to the surrounding environment [1].

Olfaction, regarded as a significant sensory perception, has been suggested to be influenced by the circadian clock based on numerous animal studies. Research in mammals [3,4] has indicated that the olfactory bulb could function as an independent circadian oscillator, manifesting as rhythmic variations in odor sensitivity. In insects, such as in cockroaches, circadian rhythms have also been observed in various aspects related to olfaction, such as the olfactory response of the antenna, olfactory sensiti-

vity, and olfactory learning and memory [5–7].

However, for humans, only limited studies have shed light on the topic. In the early and middle 20th century, a few studies indirectly indicated the possibility of circadian rhythm on human olfactory function. For example, the first related study revealed that olfactory acuity varied diurnally in accordance with food intake. Among subjects who ate lunch, acuity was found to decrease shortly after lunch, and then increase to pre-lunch levels in the later afternoon. Among participants who skipped lunch, however, diurnal variability was not observed [8]. By the end of 20th century, Lötsch et al., [9] explored chemosensitivity circadian variation in a small sample of 5 men and found that the H_2S thresholds was highest at 04:00 h and lowest at 12:00 h and 16:00 h among the 6 test timepoints of a day. Their follow-up study involved recordings of olfactory and trigeminal processing using event-related potentials (ERPs) in response to H_2S in 5 healthy men at different times of day [10]. A diurnal pattern was observed, with ERP amplitudes largest at 16:00 and 20:00 h and smallest at 04:00 h. More recently, in a study of 37 adolescents, olfactory threshold was measured using a validated forced desynchrony protocol, which allowed for the separation of circadian timing from the influence of time of day. The results showed that olfactory sensitivity was highest during the biological night (approximately 21:00 h) and lowest during the daytime [11].

Taken together, these previous studies have suggested that human olfactory function exhibits circadian variation. However, the exact olfactory-circadian pattern, including the specific timing of the lowest and highest olfactory function, remains inconsistent and inconclusive based on existing studies. On one hand, this could be limited by the small sample sizes and populations in most of the studies. For instance, Herz et al. [11], tested olfactory sensitivity in 37 adolescents, which cannot be generalized to an adult population. Similarly, Lötsch et al. [9], tested only 5 men participants, making it difficult to draw robust conclusions from their results. On the other hand, there were some studies provided only indirect evidence. For example, Goetzl et al. (8), focused on the effect of food intake on olfactory acuity variation, while Gilbert et al. [12] measured participants' nasal airflow for 8 hours, but their aim was to confirm the nasal cycle phenomenon. In addition to the variation pattern, factors that might modulate these olfactory-circadian changes also remain unexplored. Specifically, factors that associate with an individual's circadian clock, such as chronotype, sleep architecture, the effect of light, vital signs like blood pressure, and factors that relate to the sense of smell, such as age, gender, caloric intake/satiety, BMI, nasal patency, attentional level, were mentioned in previous researches as potential factors that may interplay with the relationship between olfaction and the time of day [11], but little research has been done as yet.

The relationship between olfactory performance and time of day could be essential for accurate olfactory function testing [13] and development of olfactory treatment, e.g., determining the best time for nasal administration of drugs or daily olfactory training. Considering this, the present study aimed to use a prospective design, investigate whether there is a diurnal variation of olfactory function in healthy adults, while also examining potential factors that may modulate such variation.

Materials and methods

Participants

We prospectively recruited healthy adult volunteers through flyers, the online network nebenan.de, and word of mouth. Inclusion criteria included (a) being at least 18 years of age, (b) being healthy, and (c) having subjectively normal smelling ability. Exclusion criteria included (a) being under 18 years of age, (b) having nightshift work or a sleep disorder, and (c) having acute or chronic inflammation of the nose. We excluded individuals with sleep disturbances and shift work due to their potential impact on internal rhythms and day-night/light-dark rhythms [14,15]. Sample size was determined using the G-power 3.1 software (Heinrich Heine University Düsseldorf, Düsseldorf, Germany), with the estimated minimum sample size of 36. See Supplement.

At the beginning of the study, participants were provided with detailed verbal and written information about aims and potential risks of the study. Written consent was obtained from each participant. Participants were free to withdraw from the study at any time. Data collection took place between April 2021 and May 2022 at Smell and Taste Clinic of the Department of ORL, TU Dresden, and was approved by the Ethics Committee at the University Clinic Dresden prior to the start of the study. All procedures were conducted in accordance with the requirements of the Declaration of Helsinki. Participants received a modest financial compensation for their participation.

Procedure

This is a prospective designed study. Healthy participants underwent four rounds of testing in a single day to assess their diurnal changes of olfactory function. Four repeated measurements were taken in the morning (8:00-10:00 h), at noon (12:00-14:00 h), in the afternoon (16:00-18:00 h), and in the evening (20:00-22:00 h), covering the typical waking hours of a day. However, we did not include a measurement in midnight (04:00 h) due to practical constraints and to ensure a manageable task burden for the participants. The first appointment was scheduled for 45-60 minutes per subject, whereas each subsequent appointment was scheduled for 20-30 minutes. To explore the potential sequence effects (e.g., effects of habituation or adaptation) between measurements taken subsequently on the same day, four out of the 60 participants were assigned to complete these

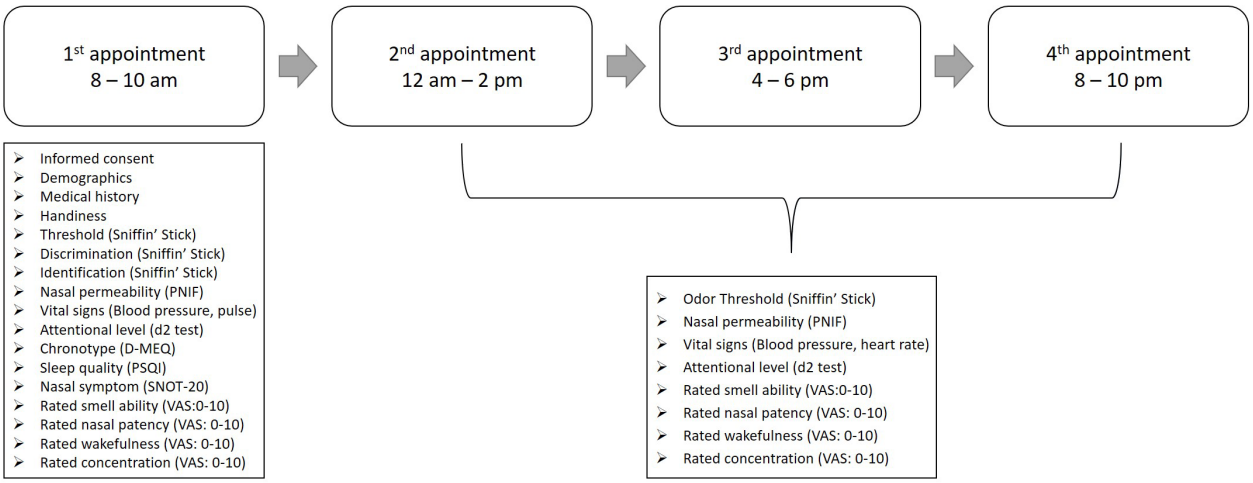


Figure 1. Study procedure. VAS = Visual analog scale, PNIF = Peak nasal inspiratory flow, D-MEQ = The German version of the Morningness-Eveningness Questionnaire, PSQI = The Pittsburgh Sleep Quality Index, SNOT-20 = The Sino-Nasal-Outcome-Test German Adapted Version.

four timepoint measurements on different days.

In the first appointment, each participant underwent a comprehensive assessment that included a full TDI (threshold, discrimination, identification) test using Sniffin' Sticks. Additionally, they were asked to fill out questionnaires that covered their medical history, nasal symptoms, sleep quality, handedness, chronotype. Besides, they rated their smell ability, nasal patency, wakefulness, and concentration level using the visual analog scale (VAS). Following this, they completed a two-minute concentration test (d2-R-test). Peak nasal inspiratory flow (PNIF), vital signs including blood pressure and heart rate were also measured for each participant. During appointments 2 (noon), 3 (afternoon), and 4 (evening), olfactory threshold test, d2-R-test, PNIF, blood pressure, heart rate, rated smell ability, rated nasal patency, rated wakefulness, and rated concentration were repeatedly measured (Figure 1).

Measurements

Olfactory function

For assessment of olfactory function, we used the validated "Sniffin' Sticks" (Burghart, Holm, Germany) [16,17]. It comprises tests for olfactory threshold (OT), discrimination (OD) and identification (OI), and allows to sum all the three dimensions to one score (TDI) that reflects the overall olfactory function. The maximum score for each test is 16, and the sum of the three tests is presented as a total TDI score (range 1-48). The higher the TDI score, the better the olfactory function. Based on normative data, we categorized participants as normosmic and hyposmic/anosmic (TDI < 30.75) [18].

Nasal patency

We objectively assessed nasal patency using a peak nasal inspiratory flow meter (PNIF ATS 97, GM Instruments, Irvine, CA, USA) [19]. This device measures inspiratory flow, producing values between 30 and 370 L/min and providing an indication of the degree of nasal obstruction. During the test, participants were instructed to hold a rubber mask tightly over their mouth and nose, and to take a deep breath through the nose only, with mouth closed, with maximum force through the mask.

Vital signs

Blood pressure and heart rate were measured using an electronic blood pressure monitor (Boso Medicus Exclusive, BOSCH + SOHN, Jungingen, Germany). To measure blood pressure, the cuff was attached to the subject's upper arm, and the subject was instructed to place the arm on the table at heart level and remain still without talking during the measurement. Blood pressure values were recorded in mmHg. Heart rate was documented in beats per minute.

Attentional level

The attention performance was assessed using the d2-Revisions-Test [20]. The test involved presenting participants with a DIN A4 sheet containing the letters "d" and "p" arranged in rows, with one to four dashes above or below them. Participants were instructed to cross out each "d" that had two dashes above or below it, or one above and one below it, while ignoring the "p" regardless of the number of dashes. To shorten the test and minimize interference, the allotted time was reduced to two minutes. The test results included the number of target objects processed, the number of omission and confusion errors, and were used to calculate concentration performance [21].

Rated smell ability/nasal patency/wakefulness/concentration

Participants assessed their sense of smell ability, nasal patency, wakefulness, and concentration using four VAS ratings at each

Table 1. Demographic information and clinical history of all participants.

	N=56		N=4	
	Mean/ Frequency	SD/ percent	Mean/ Frequency	SD/ percent
Age	31.05	12.18	37.25	11.06
Self-reported BMI	22.91	3.24	24.84	2.55
Gender				
Man	24	42.9%	2	50.0%
Woman	32	57.2%	2	50.0%
Smoke				
Yes	4	7.1%	0	0.0%
No	52	92.9%	4	100.0%
Alcohol				
Yes	34	60.7%	3	75.0%
No	22	39.3%	1	25.0%
Medication				
Yes	13	23.2%	1	25.0%
No	41	73.2%	3	75.0%
Nasal surgery				
Yes	4	7.1%	1	25.0%
No	52	92.9%	3	75.0%
Nasal polyposis				
Yes	2	3.6%	0	0.0%
No	53	94.6%	4	100.0%
Chronotype				
evening type	9	16.1%	0	0.0%
neutral type	32	57.1%	3	75.0%
morning type	14	25%	1	25%
SNOT	12.93	8.51	10.25	2.75
TDI	33.02	4.54	31.08	5.59
Olfactory function				
normosmia	40	71.4%	2	50.0%
hyposmia	16	28.6%	1	25.0%
PSQI	5.39	3.04	4.25	2.50

BMI = Body Mass Index; TDI = sum of Sniffin's Sticks Threshold, Discrimination and Identification score; SNOT = Sino-Nasal-Outcome-Test; PSQI = Pittsburgh sleep quality index.

test timepoint. The VAS is a subjective measurement tool where participants rated their perceived symptoms on a scale from 0 (no symptoms) to 10 (maximum imaginable symptom severity). The scales were labeled with contrasting terms, such as "0 [extremely sleepy] – 10 [not sleepy at all]" to provide clear endpoints for participants.

Demographics and medical history

Participants were asked about their medical history to provide a comprehensive overview of their health status. The medical history questionnaire collected general information about gender, weight, age, handedness, and substance use, such as nicotine, alcohol, and drug abuse. Additionally, the questionnaire contained specific questions regarding past nasal surgery, nasal polyposis, traumatic brain injury, asthma, other health limitations, and medications. Participants were also asked about sleep disorders and if they had participated in shift work.

Chronotype

The German version of the Morningness-Eveningness Questionnaire (D-MEQ) [22] was utilized to assess participants' chronotype. The questionnaire consisted of 19 questions that asked participants at what time of day they preferred to be active. Based on their responses, participants were classified as definite morning type, moderate morning type, neutral type, moderate evening type, and definite evening type.

Sleep quality

The Pittsburgh Sleep Quality Index (PSQI) [23] was utilized to assess the sleep habits and quality of the participants in the past four weeks. The questionnaire had seven component scores, including sleep quality, duration, efficiency, disruptive events, medication use, and daytime sleepiness. The total score ranged from 0-21 points, with higher scores indicating poorer sleep quality. The PSQI has demonstrated reliability and diagnostic validity in various studies and can be used reliably in different populations.

Nasal symptom

The Sino-Nasal-Outcome-Test German Adapted Version (SNOT-20 GAV) [24] was used to evaluate nasal symptoms in participants. This questionnaire comprised 20 questions about nasal, sinus, and general symptoms, such as nasal obstruction and sneezing. Responses were scored on a scale from 0 to 5, with a total score ranging from 0 to 100. Higher scores indicated a more severe impact of nasal symptoms.

Data analysis

Data were analyzed using SPSS 27.0 software (IBM Corp., Armonk, NY, USA). First, descriptive analyses were conducted to describe the demographfic information and study measurements of the main study group and additional exploratory group, with categorical variables shown in counts and percent, and continuous variables shown in mean ± standard deviation (SD). Then, to examine whether olfactory threshold, olfactory air flow, rated olfactory ability and rated olfactory patency varied with times of day, repeated measurement of analysis of variance (rmANOVA) were applied. Additionally, rmANOVA was also used to explore the potential interactive effect of categorical factors including age group, gender, chronotype, and olfactory function

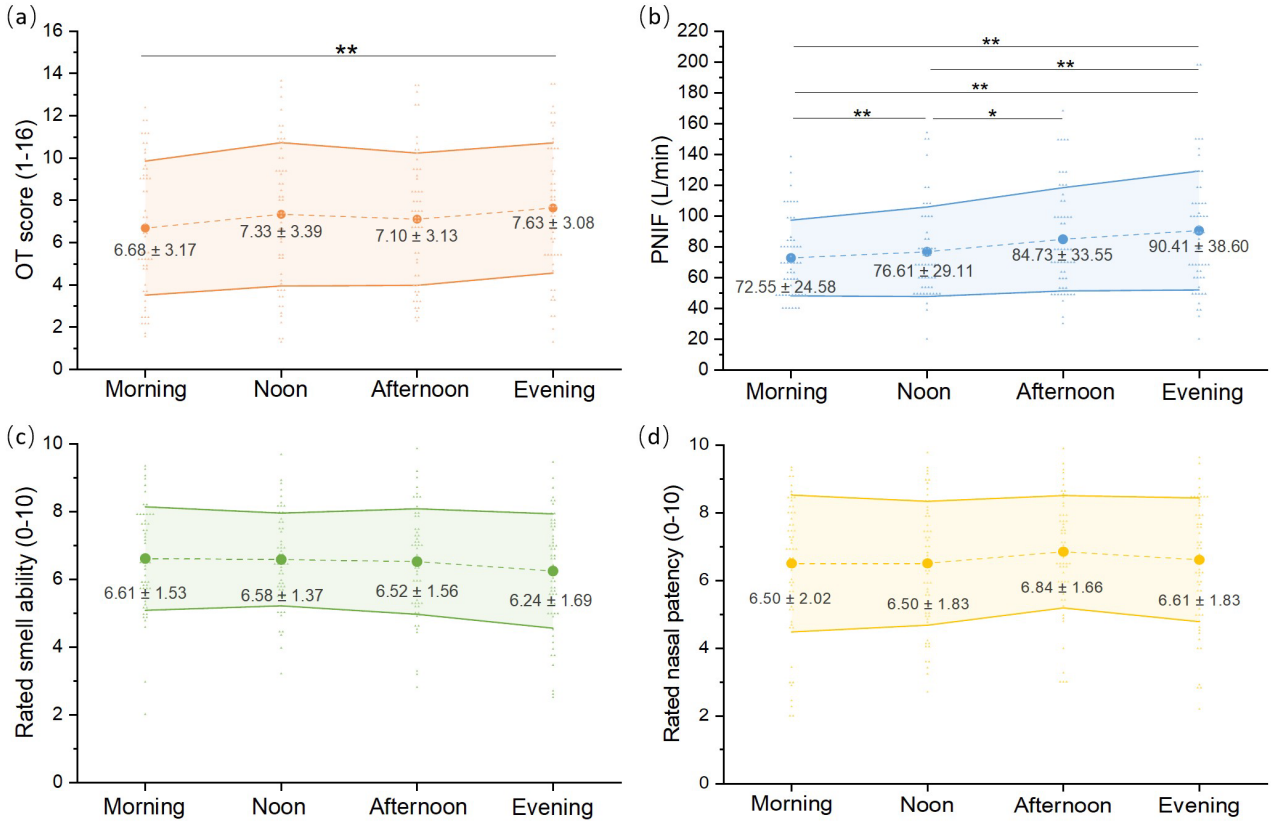


Figure 2. Olfaction throughout a day in the morning, noon, afternoon and evening. (a) Line chart of olfactory sensitivity in four testing times; (b) Line chart of objectively assessed nasal patency in four testing times; (c) Line chart of rated smell ability in four testing times; (d) Line chart of rated nasal patency in four testing times; OT = Olfactory threshold score tested by Sniffin' Sticks; PNIF = Peak nasal inspiratory flow. Means and standard deviations in four testing times are marked as dots and lower/upper solid lines. Dashed lines connecting mean values provide an estimate of the variation of olfactory function throughout a day. Data of each subject are shown in triangle points. Asterisks indicate significant results (*p 0.05, ** p < 0.01).

groups. The four timepoints of measurements served as the within-subject factor, while the mentioned categorical variables were separately treated as between-subject factors. Chronotype was categorized into morning type, neutral type, and evening type based on the above-mentioned D-MEQ questionnaire [22]. Olfactory function groups were categorized as normosmia and anosmia/hyposmia using the cutoff score (30.75) [18] from the Sniffin' Sticks test. The age group was categorized into younger group (≤40 years old) and older (>40 years old) group. We chose the age cutoff of 40 years old based on the relatively younger mean age of our sample (31.05 ± 12.18) and the distribution of participants in different age ranges. Since there were only 3 participants over 60 years old, 6 participants over 50 years old, and 11 participants over 40 years old, we decided to merge these three age ranges into a single group to increase the sample size. Furthermore, a generalized linear mixed models (GLMM) model was fit to identify factors that could potentially affect the circadian variation of olfactory threshold (the target factor). Fixed factors included age, gender, self-reported body mass index (BMI), smoking status, alcohol consumption, medication use, nasal operations, nasal polyps, chronotype, rated smell ability, rated nasal patency, wakefulness, concentration level, SNOT score, blood pressure, pulse, PNIF, TDI in baseline, D-2 test performance, PSQI score, and testing timepoint. Subject was included as a random factor. Before conducting the GLMM model, multicollinearity was examined to prevent any bias due to the significant correlations among predictors. Only predictors that met the condition of variance inflation factor <5 were included in the GLMM model. All statistical tests were 2-sided, and α=0.05 was considered statistically significant.

Results

Descriptive statistics

We included 56 participants (32 women) aged 18 to 68 years, with an average age of 31 ± 12 years old, who completed the test within one day for the main study. We also included an exploratory group of 4 participants (2 women) aged 23 to 50 years, with an average age of 37 ± 11 years, who completed the same tests on four different days. All participants reported being in good health and had a normal sense of smell. However,

Table 2. Mean and standard deviation of the study measurements in four testing times.

	Main group (N=56)				Explored group (N=4)			
	Morning	Noon	Afternoon	Evening	Morning	Noon	Afternoon	Evening
Olfactory Threshold score (1-16)								
M	6.68	7.33	7.10	7.63	6.42	7.81	5.50	5.31
SD	3.17	3.39	3.13	3.08	1.23	3.58	2.84	1.89
PNIF (L/min)								
M	72.55	76.61	84.73	90.41	81.67	72.50	67.50	72.50
SD	24.58	29.11	33.55	38.60	27.54	26.30	28.43	29.58
D2 test (KL)								
M	88.09	99.04	100.23	105.32	89.00	91.00	91.50	96.75
SD	21.61	22.88	26.39	24.40	11.53	14.31	12.37	8.30
Rated smell ability (VAS: 0-10)								
M	6.61	6.58	6.52	6.24	7.33	6.85	7.00	5.50
SD	1.53	1.37	1.56	1.69	1.70	2.21	2.21	2.04
Rated nasal patency (VAS: 0-10)								
M	6.50	6.50	6.84	6.61	7.13	7.05	6.95	5.33
SD	2.02	1.83	1.66	1.83	1.68	2.44	2.25	2.12
Rated wakefulness (VAS: 0-10)								
M	6.14	6.55	6.40	5.45	9.43	8.30	5.80	5.50
SD	2.50	2.00	1.87	2.41	0.74	1.36	2.04	1.63
Rated concentration (VAS: 0-10)								
M	6.50	6.39	6.00	5.43	8.90	8.10	5.43	5.10
SD	1.86	1.77	2.06	2.15	1.65	1.52	1.74	1.68
SBP (mmHg)								
M	130.89	132.00	131.34	132.39	126.67	129.75	126.25	134.50
SD	16.30	15.92	17.64	15.24	8.14	2.63	9.25	12.01
DBP (mmHg)								
M	80.96	82.77	81.11	81.68	78.00	77.25	78.25	83.75
SD	11.67	14.27	11.41	11.68	12.00	6.85	9.84	8.06
Pulse pressure (mmHg)								
M	49.93	49.23	50.23	50.71	48.67	52.50	48.00	50.75
SD	12.85	12.96	13.29	9.18	5.51	5.45	5.48	14.73
Pulse (beats/minute)								
M	72.15	75.96	73.95	74.07	61.67	72.75	70.00	78.00
SD	12.19	14.24	11.64	11.79	8.08	7.93	12.88	14.58

PNIF = Peak nasal inspiratory flow; D2-test = an established procedure for recording attentional performance; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; mmHg = Millimeters of mercury; M = mean; SD = standard deviation.

16 (28.6%) participants had a TDI score lower than the normosmics cut-off (30.75), with an averaged TDI score of 27.19 ± 2.77, and ranging from 19.73 to 30.25. None of them had a TDI score lower than the anosmic cut-off score (16). The rest of 40 (71.4%) participants had the averaged TDI score of 35.36 ± 2.56, ranging from 31.25 to 41.50. Other demographic information and clinical history of the investigated groups are shown in Table 1. Mean and standard deviation of the study measurements are described in Table 2.

Olfaction varied throughout the day

Objective measurement - Olfactory threshold (Sniffin' Stick Threshold test)

Repeated measurement ANOVA showed that there was a significant difference of Sniffin' Sticks OT scores between four test time points in the morning (6.68 ± 3.17), noon (7.33 ± 3.39),

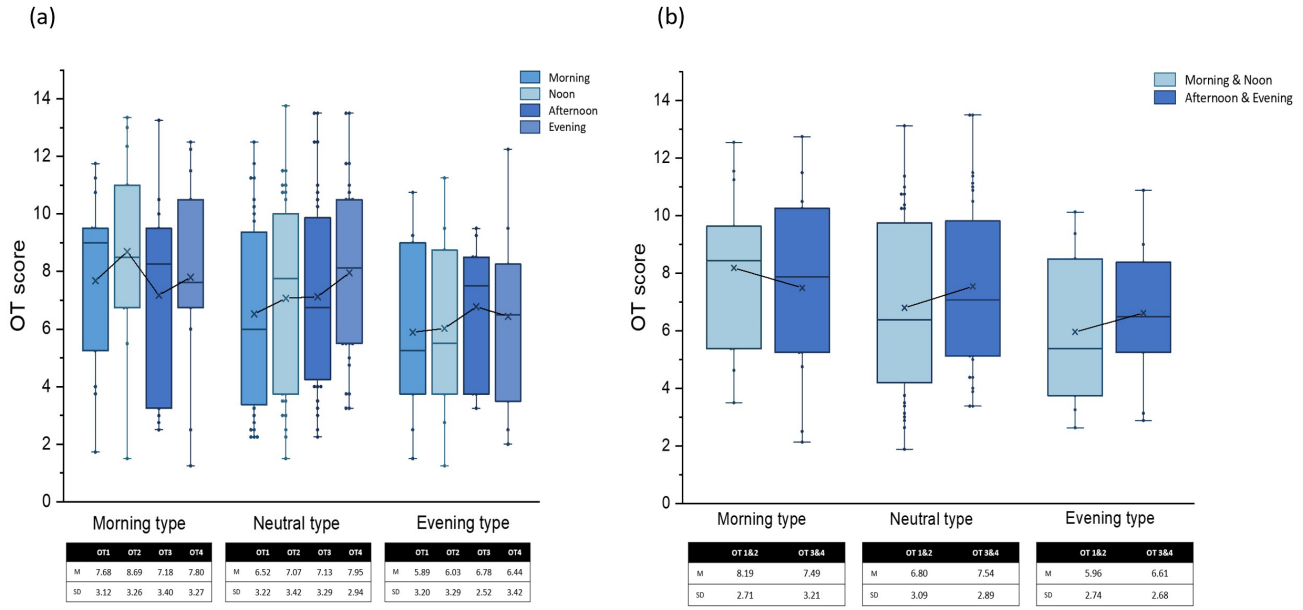


Figure 3. Olfactory Threshold throughout a day in participants with different chronotypes. The box plots display the olfactory threshold scores for different chronotype groups across four testing times (a) or two averaged testing time "morning & noon" vs. "afternoon & evening" (b). The boxes indicate the interquartile range, with a horizontal line representing the median value and a cross representing the mean value. Values within upper and lower whiskers are highest and lowest data points in the data set excluding any outliers. Data of each subject are shown in dots; M = mean; SD = standard deviation; OT 1, 2, 3 and 4 indicate olfactory scores in the morning, noon, afternoon, and evening.

afternoon (7.10 ± 3.13) and evening (7.63 ± 3.08) throughout a day (F = 2.70, p = 0.047). Post hoc tests further indicated that the OT score in the evening was the highest and significantly higher than the OT score in the morning (mean difference [MD] = 0.95, p < 0.01). While OT scores at noon and in the afternoon was higher than the OT score in the morning (MD[noon] = 0.64, p[noon] = 0.09; MD[afternoon] = 0.42, p[afternoon] = 0.24) and lower than the OT score in the evening (MD[noon] = 0.30, p[noon] = 0.37; MD[afternoon] = 0.53, p[afternoon] = 0.90), none of the individual comparisons reached the level of statistical significance (Figure 2).

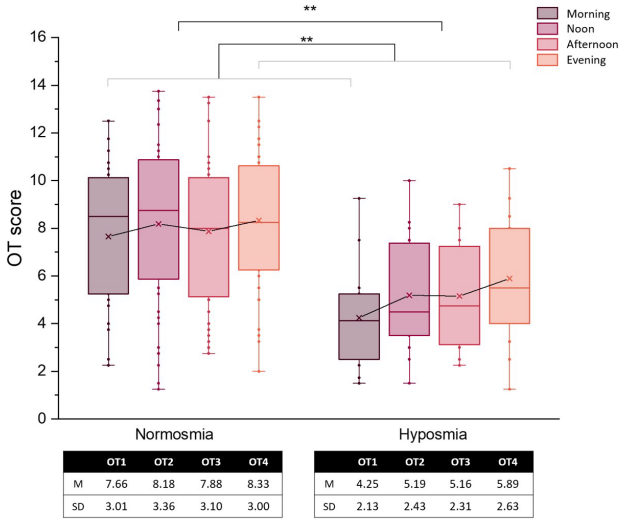
Objective measurement - nasal airflow (peak nasal inspiratory flow, PNIF)

There was a significant difference of PNIF values between four time points in the morning (72.55 ± 3.29 L/min), noon (76.61 ± 3.89 L/min), afternoon (84.73 ± 4.48 L/min) and evening (90.41 ± 5.16 L/min) during a day (F = 14.38, p < 0.01). Post hoc tests further showed that PNIF values at noon (MD = 4.05, p = 0.045), afternoon (MD = 12.18, p < 0.01) and evening (MD = 17.86, p < 0.01) were significantly higher than the value in the morning. PNIF in the afternoon (MD = 8.13, p = 0.02) and evening (MD = 13.80, p <0.01) were significantly higher than the value at noon. However, there was no significant difference of PNIF between afternoon and evening time (MD = 5.68, p = 0.69) (Figure 2). Due to the close relationship between nasal airflow and olfactory function reported in previous studies, we conducted correlation analyses between changes of the two variables. Results indicated that there were no significant correlations between OT score and PNIF in all four-time testing (r morning = 0.23, p = 0.09; r noon = -0.02, p = 0.87; r afternoon = -0.03, p = 0.85; r evening = -0.02, p = 0.89). In addition, we analyzed the correlation between the difference of OT scores between morning and evening (OT evening – OT morning) and the difference of PNIF values between morning and evening (PNIF evening – PNIF morning). There was no significant correlation between OT evening – OT morning and PNIF evening – PNIF morning (r = 0.02, p = 0.87). To further explore the relationship, we calculated the odds ratio for individuals with strong fluctuations in PNIF (PNIF evening - PNIF morning) compared to those with low fluctuations with regard to their corresponding olfactory function. Additionally, we conducted comparisons between the high and low PNIF fluctuation groups concerning TDI scores and (OT evening - OT morning) scores. The results were shown in Supplement.

Subjective measurement - rated smell ability

There was no significant difference of rated smell ability between four testing time points in the morning (6.61 ± 1.53), noon (6.58 ± 1.37), afternoon (6.52 ± 1.56), and evening (6.24 ± 1.69) throughout a day (F = 2.42, p = 0.68) (Figure 2).

Subjective measurement – rated nasal patency



	OT1	OT2	OT3	OT4
M	7.66	8.18	7.88	8.33
SD	3.01	3.36	3.10	3.00

	OT1	OT2	OT3	OT4
M	4.25	5.19	5.16	5.89
SD	2.13	2.43	2.31	2.63

Figure 4. Olfactory threshold throughout a day in normosmic and hyposmic participants. The box plots display the olfactory threshold scores for different olfactory function groups across four testing times. The boxes indicate the interquartile range, with a horizontal line representing the median value and a cross representing the mean value. Values within upper and lower whiskers are highest and lowest data points in the data set excluding any outliers. Data of each subject are shown in dots; M = mean; SD = standard deviation; OT 1, 2, 3 and 4 indicate olfactory scores in the morning, noon, afternoon, and evening.

There was no significant difference of rated nasal patency between four testing time points in the morning (6.50 ± 2.02), noon (6.50 ± 1.83), afternoon (6.84 ± 1.66), and evening (6.61 ± 1.83) throughout a day (F = 1.73, p = 0.16) (Figure 2).

Factors that may influenced the circadian rhythm of olfactory sensitivity

RmANOVA

Several categories (Gender, Age group, Chronotype, and olfactory disorder) that closely related to the study were exploratory included in the Repeated measurement ANOVA models to examine the potential interactive effect with time.

Gender. There was no significant interactive effect of gender × time (F = 1.99, p = 0.12) and main effect of gender (F = 0.65, p = 0.42). However, the main effect of time changed to a tendency towards significance (F = 2.61, p = 0.053).

Age. We divided the age in two groups, an older group with an age of more than 40 years old and a younger group with an age of no more than 40 years old. There was no significant interactive effect of age × time (F = 0.78, p = 0.51), main effects of time (F = 2.21, p = 0.09), and age (F = 0.83, p = 0.37).

Chronotype

Due to the limited sample sizes in some chronotype groups, we combined definitive evening type (n = 2) and moderate evening (n = 7) type to evening type (n = 9), and combined moderate morning type (n = 13) and definitive morning type (n = 1) to morning type (n = 14) before conducting a rmANOVA, while the neutral type remained the same (n = 32). There was no significant interactive effect of chronotype × time (F = 1.43, p = 0.21), main effect of time (F = 1.23, p = 0.30) and main effect of chronotype (F = 0.84, p = 0.44). Notably, even though the results did not reach the level of significance, there was a tendency when it came to post hoc group comparisons that the morning type group (Mean ± SE, 7.84 ± 0.75) exhibited a better OT performance than the evening group (6.29 ± 0.94). And in the morning type group, OT performance was relatively better in the morning (7.68 ± 0.85) and noon (8.69 ± 0.90) time compared to afternoon (7.18 ± 0.86) and/or evening time (7.80 ± 0.83). While in evening type group, OT performance was better in the afternoon (6.78 ± 1.07) and evening (6.44 ± 1.03) than in the morning (5.89 ± 1.06) and noon (6.03 ± 1.12) (Figure 3a).

To further examine the tendency, we averaged the OT scores of morning and noon to one score (morning OT), and the OT scores of afternoon and evening to one score (evening OT). We re-analyzed the dataset and found a significant interaction between the time of day and chronotype (F = 3.42, p = 0.04). Simple effect analysis revealed that for evening-types (n = 9), OT scores were higher in the evening (6.61 ± 2.68) than in the morning (5.96 ± 2.74), but the result did not reach the statistical significance (p = 0.27). Neutral-types (n = 32) also exhibited a similar tendency, with OT scores significantly increased in the evening (7.54 ± 2.89) compared to the morning (6.80 ± 3.09, p = 0.02). However, morning-types had, on average, higher OT scores in the morning (8.19 ± 2.71) than in the evening (7.49 ± 3.21, p = 0.14) (Figure 3b).

Hyposmia vs. normosmia

There was no significant interactive effect of olfactory function group × time (F = 0.59, p = 0.62). However, there was a significant main effect of olfactory function group (F = 15.61, p < 0.01) and time (F = 3.15, p = 0.03). OT score was significantly higher in the normosmia group than in the hyposmia group (MD = 2.89, p <0.01). OT score was significantly higher in the evening than the morning (MD = 1.16, p < 0.01). See Figure 4.

Generalized Linear Mixed Models (GLMM)

As shown in Table 3, participants had significantly lower OT scores in the morning compared to when they were tested in the evening (β = -1.33, SE = 0.48, p = 0.02). Participants with an evening chronotype had a significantly lower OT score compared to those with a morning chronotype (β = -2.18, SE = 1.00, p = 0.03). TDI score in baseline also significantly predicted the OT performance across time (β = 0.40, SE = 0.07, p < 0.01). Moreover, as self-reported BMI increased, participants had significantly higher OT scores (β = 0.23, SE = 0.09, p = 0.02). Participants

Table 3. Factors that predicted olfactory sensitivity based on the GLMM model.

Model term	β	SE	t	p	95%CI	
					Lower	Upper
Time						
Morning	-1.33	0.48	-2.77	<0.01	-2.28	-0.38
noon	-0.52	0.42	-1.25	0.21	-1.34	0.30
afternoon	-0.40	0.35	-1.15	0.25	-1.08	0.28
evening	Reference					
Rated smell ability (0-10)	0.38	0.17	2.24	0.03	0.05	0.72
Rated nasal patency (0-10)	-0.56	0.16	-3.48	<0.01	-0.88	-0.24
Chronotype						
Evening	-2.18	1.00	-2.17	0.03	-4.16	-0.19
Neutral	-0.91	0.68	-1.33	0.19	-2.26	0.44
Morning	Reference					
Self-reported BMI	0.23	0.09	2.44	0.02	0.04	0.41
Age	-0.03	0.03	-0.90	0.37	-0.09	0.03
Gender						
Man	-0.69	0.61	-1.13	0.26	-1.90	0.52
Woman	Reference					
Smoke						
Yes	2.76	1.68	1.65	0.10	-0.55	6.07
No	Reference					
Alcohol						
Yes	0.33	0.70	0.47	0.64	-1.06	1.71
No	Reference					
Medication						
Yes	-0.55	0.71	-0.78	0.44	-1.96	0.85
No	Reference					
Nasal surgery						
Yes	-0.74	1.40	-0.53	0.60	-5.30	2.03
No	Reference					
Nasal polyposis						
Yes	1.20	1.93	0.62	0.54	-2.62	5.02
No	Reference					
Wakefulness (0-10)	-0.12	0.11	-1.03	0.30	-0.34	0.11
Concentration (0-10)	0.16	0.14	1.09	0.28	-0.13	0.44
SNOT	0.02	0.04	0.43	0.67	-0.06	0.09
SBP	-0.003	0.02	-0.20	0.85	-0.04	0.03
DBP	-0.02	0.02	-0.77	0.44	-0.06	0.03
Pulse	0.01	0.02	0.40	0.69	-0.03	0.04
PNIF	-0.01	0.01	-1.66	0.10	-0.03	0.002
TDI	0.40	0.07	4.98	<0.01	0.22	0.51
KL (d2 test score)	-0.003	0.01	-0.20	0.85	-0.03	0.03
PSQI	-0.15	0.12	-1.32	0.18	-0.38	0.08

BMI = Body Mass Index; TDI = sum of Sniffin's Sticks Threshold, Discrimination and Identification score; SNOT = Sino-Nasal-Outcome-Test; PSQI = Pittsburgh sleep quality index; PNIF = Peak nasal inspiratory flow; D2-test = an established procedure for recording attentional performance; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; mmHg = Millimeters of mercury; β = regression coefficient; SE = standard error; CI = Confidence interval.

reporting higher rated smell ability had a significantly higher OT score (β = 0.38, SE = 0.17, p = 0.03). However, as rated nasal patency increased, OT score decreased (β = -0.56, SE = 0.16, p < 0.01). Additionally, subject factors showed significant individual differences (β = 2.16, SE = 0.82, p < 0.01).

Discussion

The present study sheds light on the relationship between the olfactory function and the internal clock in human adult, and possible factors that may influence such a relationship. Our findings confirm our initial assumption that the olfactory function fluctuates throughout the typical waking hours of the day, with the most sensitive threshold and best nasal airflow occurring in the evening before sleeping time. Interestingly, it appears that the circadian changes of nasal airflow may not significantly depend on the circadian changes of the olfactory sensitivity. Our results also suggest that the variation may be regulated by factors such as chronotype and BMI. These findings support the idea that the circadian rhythm plays a role in regulating olfactory function.

Olfaction varied throughout the day

First and foremost, we observed that a significant change in olfactory sensitivity throughout the waking hours of the day, with the highest threshold scores found in the evening and the lowest scores found in the morning. These results implied that olfactory sensitivity could be a function of circadian rhythm. And the results were similar to previous studies in adolescents (12-16 years old) which suggested that better olfactory sensitivity occurred in the evening before sleeping time [11]. Some evolutionary biological reasons may contribute to our observation. For example, using senses that do not rely on daylight to detect and respond to danger in darkness. In addition, many species reproduce at the end of the day, and the sense of smell influences mate choice. Moreover, anthropological records suggest that the evening meal was historically considered the most important meal of the day, and since olfaction plays a major role in food selection and enjoyment, it could be assumed that olfaction would be more prominent at this time. On the other hand, it could also be hypothesized that the most important meals were held at times when the sense of smell is most accurate [11]. The finding has important research and clinical implications. For example, it could be recommended to test olfactory threshold in the morning to decrease the possibility of missed diagnosis [13]. The knowledge could be also beneficial in clinical treatment. For example, olfactory training might be most effective when performed in the evening. Interestingly, although there was a significant difference of olfaction between morning and evening, the changes were subtle in terms of absolute value and went unnoticed by the participants, as there were no significant differences in their rated smell ability across different testing times. Such discrepancies between psychophysiological measurements and self-reports can be understood from a biological and evolutionary perspective. As a normal physiological function, the biological clock may guide the olfactory system to be more effective at specific times of the day. For example, the sense of smell may be heightened during mealtime, increasing our appetite, and during the night, promoting social interactions and mating behaviors. However, these fluctuations in olfaction should be small enough to prevent sensory overload, ensuring that our sensory system can process and adapt without overwhelming our senses. In addition, the observed discrepancy between self-reports and psychophysiological measurements of olfaction has also been observed in clinical practice and previous studies. It has been reported that individuals with undetectable olfactory loss may experience less impact on quality of life [25].

In the present study, we included participants with a subjective perception of a normal sense of smell and without diagnosis of any olfactory disorder. However, there were still some participants who could be categorized as hyposmics (n=16, 29%) based on the Sniffin' Stick normative data. The rmANOVA indicated that both normosmic and hyposmic participants exhibited a similar olfactory-circadian tendency during the waking hours, with better olfactory performance in the evening and poorer performance in the morning. This result seems to imply that patients with an olfactory disorder may also exhibit a similar pattern. However, it is necessary to obtain data from patients with confirmed diagnoses as olfactory disorders resulting from various causes, such as sinonasal disease, head trauma, or viral infections [26], that may also exhibit fluctuations over time and potentially display a circadian pattern [27]. In a study by Landis et al. [27], a 27-year-old female with chronic rhinosinusitis reported a peculiar pattern of olfactory function: it appeared an hour after breakfast, was normal during the daytime, and disappeared in the early evening. The researchers hypothesized that this pattern could be attributed to mucosal congestion in the nasal and ethmoid cavities, influenced by the endogenous release of cortisol. The cortisol levels have been shown to typically peak in the early morning, remain stable throughout the day, and decline in the late afternoon and evening, which closely paralleled the patient's symptoms [28,29]. Moreover, their successful and sustained cortisol treatment in this patient provided further support to the hypothesis. Thus, expanding the participant pool to include patients with olfactory disorders of various causes in future study is essential for a comprehensive understanding of the olfactory-circadian pattern and providing adequate treatment strategies.

Additionally, nasal patency showed a similar pattern as olfactory sensitivity. It was revealed that PNIF scores gradually improved over the course of a day of the waking time, with the highest scores in the evening and the lowest scores in the morning, suggesting diurnal variations in nasal function similar to olfactory

sensitivity. While the exact mechanism remains uncertain, one potential explanation could be associated with the influence of the circadian rhythm of some physiological processes. For instance, the human body has been shown to have the lowest body temperature in the early morning and the highest in the late afternoon or early evening [30,31]. Moreover, as physical activity may increase from the morning after being awake to the evening before sleeping [32], it is possible that the breathing rate also rises and helps to widen the nasal passages. The circadian pattern of nasal airflow may have clinical implications for disease management and nasal surgery planning. For example, in allergic rhinitis patients, preventive measures like allergen avoidance, nasal irrigation, and timed medication administration may help to minimize symptoms during peak hours. Understanding the circadian rhythm of nasal airflow may be beneficial for scheduling nasal surgery. Since nasal airflow is commonly lowest in the morning, scheduling surgery for later in the day might provide a better chance for optimal post-operative nasal airflow and patient comfort.

In addition, although previous studies revealed a close relationship between nasal airflow and olfactory function [33,34], our data did not show statistically such significant correlations between OT scores and PNIF in all four sessions. However, this could be due to the fact that olfactory function is non-linearly correlated with nasal airflow [34–36]. Specifically, adequate nasal airflow can enhance the perception of odors, but extremely small or large nasal airflow may decrease the perception of odors. Such a tendency was also observed in the scatter plots of OT scores and PNIF values (see Supplement). However, when we further explored the relationship between [OT evening – OT morning] and [PNIF evening – PNIF morning], no significant correlation was detected. Additionally, there was no significant odds ratio observed between participants with strong PNIF fluctuation and those with low PNIF fluctuation. There were also no significant differences found in TDI scores and [OT evening – OT morning] scores between participants with strong PNIF fluctuation and those with low PNIF fluctuation (See Supplement). These findings collectively indicate that the circadian changes in nasal airflow may not be significantly associated with the circadian changes in olfactory sensitivity. However, it is important to note that the absence of a significant association does not completely exclude the possibility of a causal or significant interaction between circadian changes in nasal airflow and olfactory sensitivity. The limitations of the PNIF test as a precise measure of nasal airflow could have influenced these results [37]. Considering the use of alternative methods such as rhinomanometry or acoustic rhinometry [38,39] may help to further investigate and confirm the relationship.

Factors that may influence the circadian variation of olfactory sensitivity

Based on the results of rmANOVA and GLMM, it was identified that several factors, including chronotype, self-reported BMI, rated smell ability, and rated patency, may regulate the relationship between olfactory sensitivity and the circadian clock.

First and foremost, our results, for the first time, indicated that chronotype may contribute to individual variability in olfactory sensitivity. Specifically, participants with an evening chronotype had significantly lower OT scores than those with a morning chronotype. This finding is consistent with several prior researches indicating that a large number of physiological markers such as the sleep-wake cycle [40], melatonin [41], cortisol [42], and activity levels [43] peak earlier in morning-types than in evening-types. This is likely due to differences of chronotype in the timing of the circadian rhythm, which affects the activity of olfactory receptor cells in the nose. With caution, chronotype could also explain why the variation pattern of olfactory sensitivity in the exploratory group (4 participants who were tested in different days) exhibited different tendency than the main groups in our current study, with higher OT scores in the morning and noon, and lower OT scores in the afternoon and evening (Table 2). As shown in Table 1, all the four participants were morning-types or neutral-types. This finding has important implications for understanding the factors that influence olfactory function and potentially for olfactory function rehabilitation. By targeting chronotype changes, such as through chronotherapy or light exposure, it might be possible to optimize olfactory function in individuals, particularly in children and teenagers whose chronotype may still be developing [44]. However, further research with larger sample sizes and diverse populations is necessary, as our rmANOVA only detected the interactive effect between chronotype and time when combining OT scores in the morning and noon, and in the afternoon and evening, even though the GLMM revealed the significant difference between morning-types and evening-types.

The results of the GLMM model indicated that higher self-reported BMI was associated with better olfactory OT scores across the four timepoints. This finding is consistent with some previous studies suggesting that olfactory sensitivity increases with increasing BMI [45,46]. However, it should be noted that opposite trends have been reported more commonly in the literature [47,48]. Taken together, olfactory sensitivity may not change linearly with BMI, but instead may exhibit an inverted U-shaped curve. Specifically, individuals in the middle of the BMI range may have normal olfactory sensitivity, while those on the extremes may exhibit olfactory sensitivity deficits [49]. It is worth noting that the participants in our study had relatively low self-reported BMI, with an average self-reported BMI of 22.91 ± 3.24 and a range from 18.87 to 38.28. Therefore, it is possible that many of those with higher self-reported BMI in our sample are still within the normal BMI range, which could explain why higher self-reported BMI was associated with better olfactory sensitivity in our study.

Our study found that self-rated smell ability was positively associated with olfactory sensitivity across the four time points tested, which is consistent with some previous research [33]. This may imply that healthy participant could largely assess their actual olfactory sensitivity variation among a day. However, unexpectedly, the GLMM detected a negative association between the rated nasal patency and olfactory sensitivity. Further analysis with Pearson correlations and scatter plots showed a significantly negative correlation between rated nasal patency and olfactory sensitivity in the afternoon (see Supplement). This is contrary to previous studies and clinical observations which suggest that higher self-ratings of nasal patency are associated with increased olfactory sensitivity [33]. The reason for this conflicting result is unclear and highlights the need for further research to clarify the relationship between self-rated nasal patency and olfactory sensitivity.

There are several limitations of this study that should be discussed. Firstly, due to practical constraints and to ensure that participants' task burden was manageable, our study was limited to four timepoints within the waking hours of 8:00 h to 22:00 h. We did not measure during the middle of the night (04:00), which resulted in incomplete coverage of the entire 24-hour cycle. Consequently, it becomes challenging to generalize the findings beyond the waking hours and compare them directly to studies that encompassed the entire circadian cycle. Therefore, the conclusions drawn from our study are specific to the waking hours of the day. Future studies should address this limitation. Secondly, we designed that subjects would undergo olfactory function testing four times within a single day. While this design helped to control for daily variability in factors such as emotional state and life events, it may have introduced a learning effect in some tests, such as the test of attention (d2 test). To address this concern, we conducted an additional analysis with four additional subjects tested on four different days at various times of the day. While this exploratory analysis provided some insight, the sample size of four is too small to exclude biases. Therefore, future studies with larger sample sizes and more diverse populations, such as patients with olfactory disorders from various causes, are needed to confirm these findings. Thirdly, due to the current observational study design, we were unable to establish causality. To address this limitation, a randomized controlled design with a condition that deliberately disrupts the circadian rhythm (such as sleep deprivation) should be considered in future studies. Besides, since the calculation of BMI was based on self-reported height and weight, there is a possibility of accuracy implications that could potentially impact our results. It is important to incorporate objectively measured BMI in the analysis in future study. Finally, some potentially confounding factors that may influence olfaction or/and circadian clock, such as hunger or satiety [50,51], hormonal state (endogenous steroid release) [52], and menstrual cycle [53,54] were not tested.

Conclusion

Our study provides evidence that the circadian clock significantly influences olfactory function in adult humans. Olfactory sensitivity varies throughout the waking time of the day, with the highest olfactory sensitivity observed in the evening and the lowest olfactory sensitivity in the morning. This varied pattern was also seen in nasal patency. Interestingly, it appears that the circadian changes of nasal airflow may not significantly depend on the circadian changes of the olfactory sensitivity. In addition, chronotype and BMI may play a role in regulating the circadian variation of olfactory sensitivity. Overall, our findings provide valuable insights into the temporal dynamics of olfactory function and highlight the multifaceted nature of its regulation. This knowledge may also provide reference for future research on the accurate diagnosis and treatment of olfactory dysfunction.

Authorship contribution

YM: data analysis and interpretation, manuscript drafting and revision, and final approval of the manuscript; MR: data collection, manuscript revision, and final approval of the manuscript; TH: study conception, data interpretation, manuscript revision, and final approval of the manuscript.

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Conflict of interest

The authors declare no competing financial interests.

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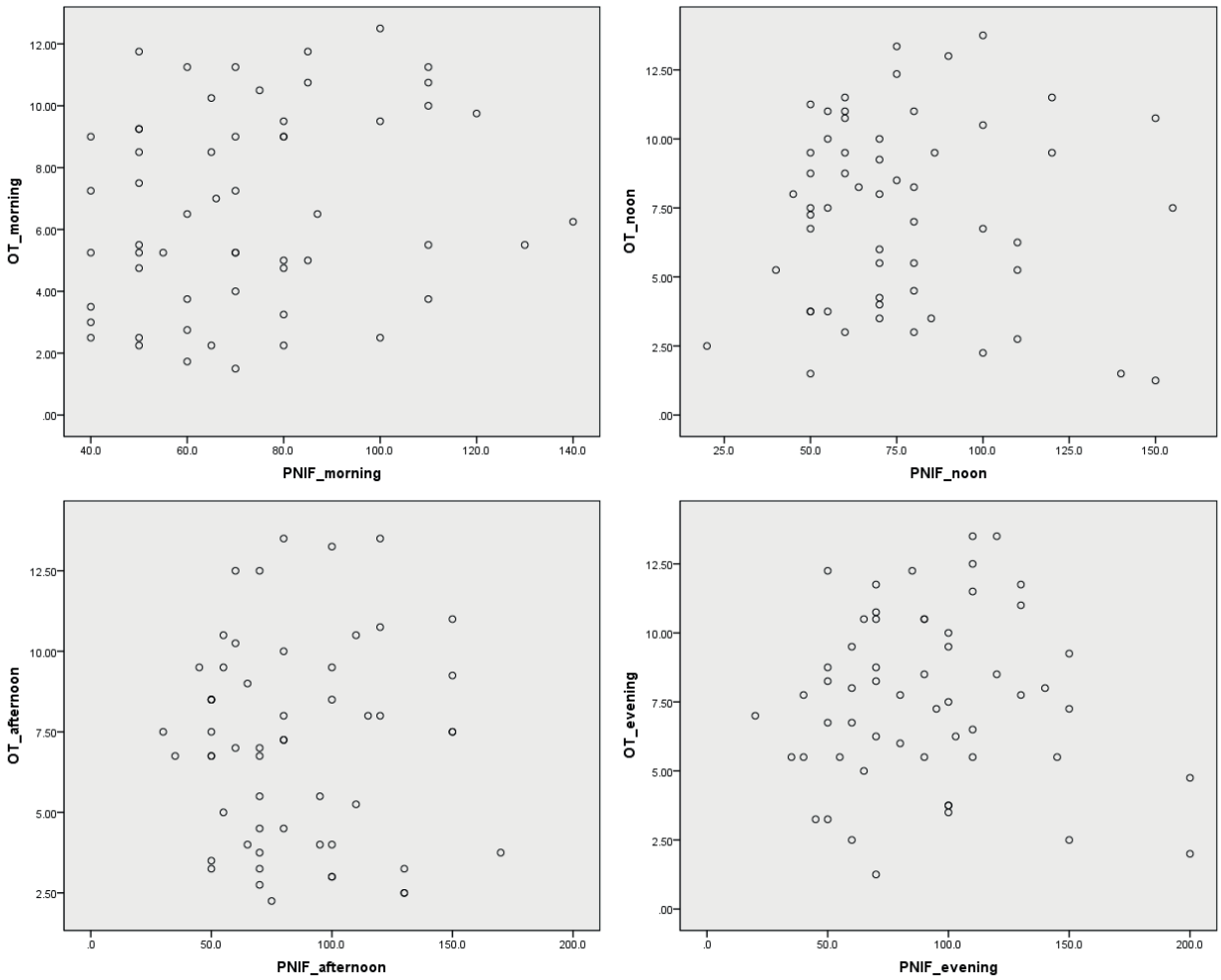
SUPPLEMENTARY MATERIAL

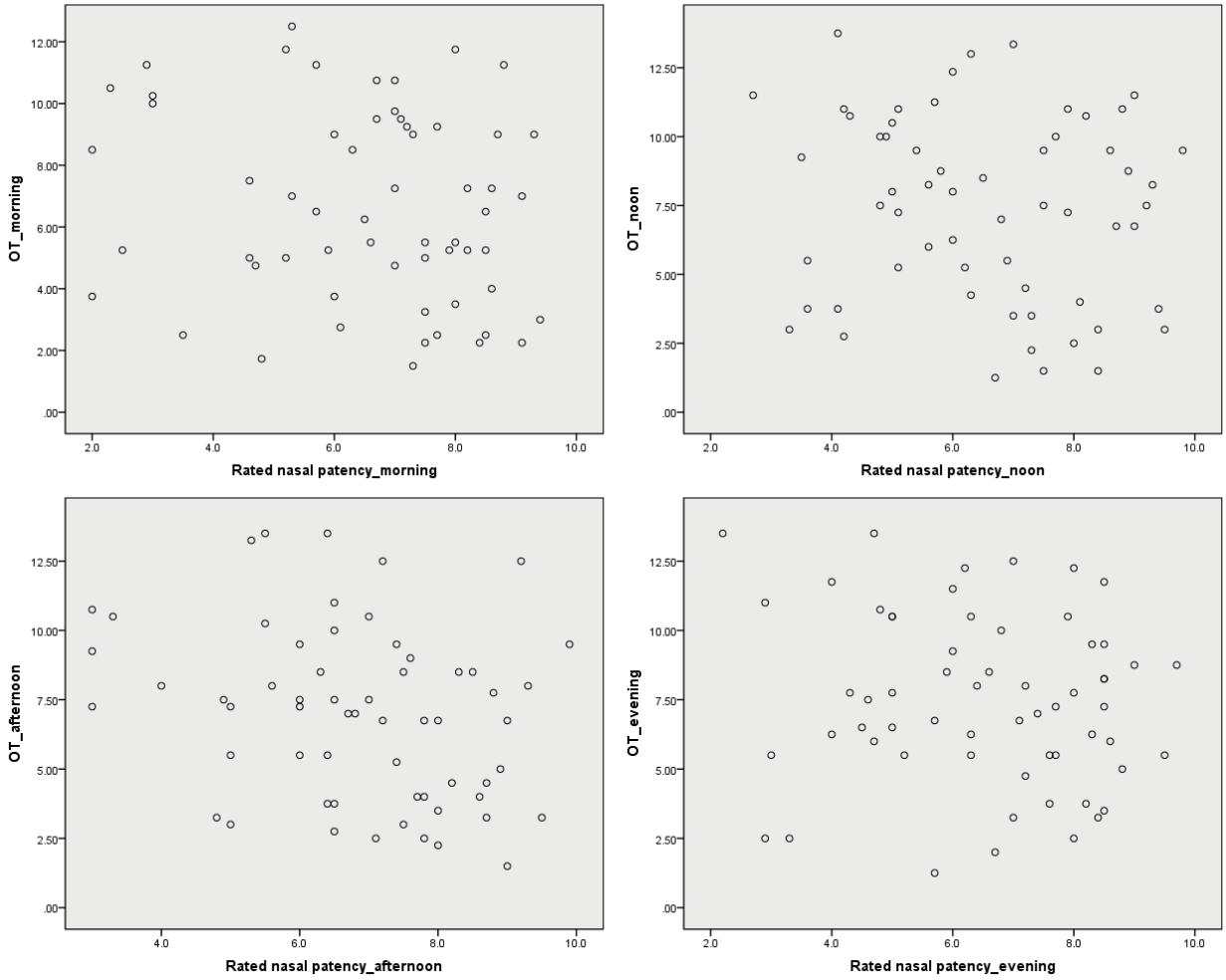
1. The correlations between measured nasal airflow and olfactory sensitivity

There were no significant correlations between PNIF and OT scores across all the four testing: $r_{morning} = 0.23$, $p = 0.09$; $r_{noon} = -0.02$, $p = 0.87$; $r_{afternoon} = -0.03$, $p = 0.85$; $r_{evening} = -0.19$, $p = 0.89$. However, a non-linear tendency was observed between the two variables. Specifically, moderate PNIF values were associated with the highest OT scores, while extremely low or high PNIF values were associated with reduced OT scores. The scatter plots below illustrate this relationship.



2. The correlations between rated nasal patency and olfactory sensitivity

There was a significant negative correlation of rated nasal patency and OT score in the afternoon ($r = -0.27$, $p = 0.04$), but there were no statically significant correlations of rated nasal patency and OT scores in the morning ($r = -0.19$, $p = 0.17$), at noon ($r = -0.12$, $p = 0.38$), and in the evening ($r = -0.20$, $p = 0.14$). Scatter plots are shown below.



3. 3. Odd ratios for PNIF fluctuation with regard to olfactory function

To assess the potential relationship between the degree of PNIF fluctuation/improvement and olfactory function (normosmic vs. hyposmics), we calculated the odds ratios. Firstly, to quantify the extent of PNIF fluctuations, we calculated the difference in PNIF between the morning and evening measurements ($PNIF_{evening}$ - $PNIF_{morning}$). Based on the data distribution of PNIF differences, we defined strong PNIF fluctuation as having a ($PNIF_{evening}$ - $PNIF_{morning}$) ≥ |40|, while low PNIF fluctuation was defined as having a ($PNIF-_{evening}$ - $PNIF_{morning}$) ≤ |5|. As a result, there were 14 participants (8 normosmics and 6 hyposmics) in the strong PNIF fluctuation category and 15 participants (12 normosmics and 3 hyposmics) in the low PNIF fluctuation category (See Table below). We found that there was no significant odds ratio between the strong PNIF fluctuation group and the low PNIF fluctuation group (OR = 3, Z = 1.31, p = 0.10). Furthermore, we conducted comparisons between the high and low PNIF fluctuation groups regarding TDI scores and ($OT_{evening}$ - $OT-_{morning}$) scores. The results of the independent t-test indicated no significant difference in TDI scores between the high PNIF fluctuation group (32.14±4.24) and the low PNIF fluctuation group (33.03±3.46, t = 0.62, p = 0.54). Similarly, there was no significant difference in ($OT_{evening}$ - $OT_{morning}$) scores between the high PNIF fluctuation group (0.71±2.79) and the low PNIF fluctuation group (2.12±3.07, t = 1.29, p = 0.21). Notably, a trend of higher ($OT_{evening}$ - $OT_{morning}$) scores was observed in the low fluctuation group. However, the direction of this tendency was inconsistent with the hypothesis that circadian changes in PNIF are positively associated with circadian changes in olfactory sensitivity.

	Low fluctuation	High fluctuation
Normosmic	12	8
Hyposmic	3	6

4. Sample size determination

To determine the sample size, we used the GPower 3.1 software (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) to conduct a power analysis. The analysis was based on the "F tests" test family, the "ANOVA: Repeated measures, within factors" statistical test, and the "A priori: Compute required sample size – given a, power, and effect size" type of analysis. We set an effect size (f) of 0.25, significance level (α) of 0.05, and desired power (1-β) of 0.95. The number of groups was 1, and the number of measurements was 4. The estimated minimum total sample size generated by the analysis was 36 participants. To ensure a higher level of certainty in our data interpretation, we ultimately included 56 participants in our study. See Figure of parameter setting below.