The Influence of Odorants on Respiratory Patterns in Sleep

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Abstract

To assess the feasibility of using odors as a potential mechanism for treating sleep apnea, we set out to test the hypothesis that odorants delivered during sleep would modify respiratory patterns without inducing arousal or wake in healthy sleepers. We used 2 mildly trigeminal odorants: the pleasant lavender and unpleasant vetiver oil and 2 pure olfactory odorants: the pleasant vanillin and unpleasant ammonium sulfide. During sleep, an olfactometer delivered a transient odorant every 9,12, or 15 min (randomized), providing 21–37 odorant presentations per night. Each of 36 participants was studied for 1 night and with 1 of the 4 different odorants tested. In addition to standard overnight polysomnography, we employed highly accurate measurements of nasal and oral respiration. Odorants did not increase the frequency of arousals or wake but did influence respiration. Specifically, all 4 odorants transiently decreased inhalation and increased exhalation for up to 6 breaths following odor onset. This effect persisted regardless of odorant valence or stage of sleep. These results suggest that the olfactory system may provide a path to manipulate respiration in sleep.

Key words: apnea, olfaction, sleep

Introduction

Sleep apnea is a sleep disorder characterized by repetitive cessation or decreased amplitude of breathing lasting 10 s or more that may occur up to hundreds of times per night (AAoSMTF 1999). The cessations of breath lead to oxyhemoglobin desaturation and often lead to an arousal (Chesson et al. 1997). The prevalence of the disorder is ~4% in middle-aged men and ~2% in middle-aged women, and an additional ~7% of the population suffer from a mild sleep apnea (Young et al. 1993; Fleisher and Krieger 2007). Apnea can result from either a central nervous system malfunction in respiratory driving (central sleep apnea, CSA) or a peripheral malfunction of upper airway collapse despite respiratory efforts (obstructive sleep apnea, OSA). CSA and OSA can materialize independently, and they can co-occur (Eckert et al. 2009).

Apnea has a significant impact on daily life as well as on general health. Clinical consequences of the disorder cover a wide spectrum including neurocognitive dysfunction (Bedard et al. 1991; Ferini-Strambi et al. 2003), cardiovascular disease (Marin et al. 2005; Eckert et al. 2009), and metabolic dysfunction (Punjabi et al. 2004). There has been limited successes in treating apnea with pharmacological

(Carley et al. 2007; Fleisher and Krieger 2007; Brunner 2008) and surgical (Charuzi et al. 1992; Liao et al. 2002) methods, and the standard treatment for apnea remains a device consisting of a pump and nasal mask that provide continuous positive airway pressure (CPAP). CPAP acts as a pneumatic splint that elevates and maintains a constant pressure along the upper airway during inspiration and expiration that prevents airway collapse (Issa and Sullivan 1984; Mortimore and Douglas 1997). The advantages of CPAP include improved sleep quality, reduced apnea symptoms such as morning headaches, dry mouth and snoring (Ballester et al. 1999), and reduced risk of cardiovascular mortality (Nelesen et al. 2001). The major disadvantage of CPAP is the relatively low compliance. Although some studies report usage as high as 88% (Pepin et al. 1995), typical numbers appear to be that ~12% of users abandon therapy after 1 night (Waldhorn et al. 1990), and only 46% used CPAP on a regular daily bases (Kribbs et al. 1993). Thus, apnea remains an often untreated disorder.

An ideal treatment for apnea would be to somehow "jump start" respiration without inducing arousal or wake. Two independent lines of evidence suggest that odors may serve

in this role. The first line of evidence is that odors do not arouse or wake. More specifically, odors can be divided into "trigeminals," so called because they simultaneously activate trigeminal as well as olfactory receptors, and "pure olfactants," so called because they activate olfactory receptors alone (Doty et al. 1978; Hummel and Livermore 2002). Whereas strong trigeminal odors may induce arousal or wake (Carskadon and Herz 2004; Stuck et al. 2007; Grupp et al. 2008), several studies have suggested that mildly trigeminal and pure olfactory odorants presented during sleep do not arouse or wake (Badia et al. 1990; Carskadon and Herz 2004; Stuck et al. 2007; Grupp et al. 2008). The second line of evidence that implicates olfaction as a possible route to treating apnea is that odors modify respiratory patterns during wake. More specifically, nasal inspirations are larger following pleasant versus unpleasant odors (Bensafi et al. 2007) or dilute versus intense odors (Warren et al. 1994; Sobel et al. 1999; Walker et al. 2001; Johnson et al. 2003). Whether odors similarly influence respiratory patterns in sleep is unclear. Badia et al. (1990) reported no influence of odors on respiration in sleep. However, respiration was not the major focus of that study and was therefore measured with relatively insensitive methods and summated over relatively long temporal windows. In turn, if odors do modify respiration in sleep as they do in wake, one can hypothesize a device that would trigger an odor at the onset of an apnea, thus jump-starting the respiratory pattern without inducing arousal or wake. Thus, here we set out to use pleasant and unpleasant mildly trigeminal and pure olfactory odorants, concurrent with highly accurate measures of nasal and oral respiration, in order to ask whether odors modify respiratory patterns during sleep without causing arousal or wake.

Materials and methods

Participants

Forty-five healthy subjects (21 women and 24 men) ranging in age from 23 to 36 years ($M = 27.2 \pm 2.28$ years) participated in the study after providing informed consent to procedures approved by the Committee for Protection of Human Subjects at the Assuta hospital. Subjects were screened for abnormal sleep habits and history of nasal insults. Exclusion criteria were irregular breathing pattern, insufficient sleeping time, and use of medication or demonstration of sleep apnea syndrome with respiratory disturbance index >10. Nine subjects failed to meet the study criteria and were therefore excluded from analysis.

Odorants

Four odorants composed of both neat molecules and blends were used: undiluted lavender oil (Sensale) ($n = 14$) considered pleasant and mildly trigeminal, undiluted vetiver oil (Givaudan) ($n = 10$) considered unpleasant and mildly trigeminal, 3% v/v vanillin (CAS 121-33-5, Sigma-Aldrich)

($n = 15$) considered pleasant and pure olfactory, and 1% v/v ammonium sulfide (CAS 12135-76-1, Sigma-Aldrich) ($n = 6$) considered unpleasant and pure olfactory.

Odorant delivery

Odorants were delivered with a computer-controlled air dilution olfactometer built according to principals we have previously described in detail (Sobel et al. 1997; Johnson and Sobel 2007). The odorant line culminated at a small nasal mask that was subserved by a vacuum line pulling at the same rate of airflow. This provided an odor environment at the nose where odorant onset and offset occurred within 2 and 260 ms, respectively, with no visual, auditory, tactile, humidity, or thermal cues as to the alteration. In other words, the odorant stimulus was not a puff of air but rather a block of odorant embedded within an airflow that was constant for the duration of the study. Importantly, the olfactometer itself was located in a room adjacent to the sleep room, and only the airflow tubing crossed into the sleep room via a wave guide within the stainless steel–coated wall. This provided additional separation from any possible visual or auditory stimulation associated with the olfactometer (e.g., LEDs on its front panel, etc.).

Polysomnography and sleep scoring

Physiological measurements were recorded using a Power-Lab 16SP Monitoring System (ADInstruments) running off a Macintosh G4 computer using a sampling rate of 1000 Hz and a 50-Hz notch filter to remove electrical artifacts. For all measures dependent on electrodes, the scalp surface was cleaned with mild abrasive gel (Nuprep gel, Aurora) in order to assist in lowering impedance at the electrode site. For pasted electrodes on the rest of the body, the skin surface was also first cleaned with alcohol. We recorded the following measures:

- Electroencephalogram (EEG) was obtained through 2 circular electrodes (0.9 mm diameter) that were located at positions C3 and C4 according to the 10–20 system and were referenced to electrodes on the opposite mastoids (A2 and A1, respectively). Signals were amplified using a preamplifier (Octal Bio Amp ML138, ADInstruments).
- Electroocculogram was obtained through 2 circular Ag/AgCl conductive adhesive electrodes (0.9 cm diameter), placed 1 cm above and laterally of each eye, and referenced to electrodes on the opposite mastoids (A2 and A1, respectively). Signals were amplified using a preamplifier (Octal Bio Amp ML138, ADInstruments).
- Electromyogram (EMG) was obtained through 2 circular Ag/AgCl conductive adhesive electrodes (0.9 cm diameter). The electrodes were located bilaterally adjacent to the submentalis muscles. Signals were amplified using a preamplifier (Octal Bio Amp ML138, ADInstruments).

Electrocardiogram (ECG) was obtained through 3 circular Ag/AgCl conductive adhesive electrodes (0.9 cm diameter). Electrodes were placed on both the left and the right sides of the abdomen, and a ground electrode was placed on the left foot. Signals were amplified using a preamplifier (Bio Amp ML132, ADInstruments).

Blood oxygination (SpO2) was measured with an oxymeter (MLT321 SpO2 Finger Clip Sensor, ADInstruments) embedded within a finger clip placed on the left index finger.

Overall respiration was computed as a reflection of changes in thoracic respiration and abdominal respiration circumference measured using 2 piezoelectric respiratory belt transducers (1132 Pneumotrace II, UFI).

Nasal and Oral respiration was measured using separate pneumotachometers (high-sensitivity flowmeter model #4719, Hans Rudolph, Inc.) that were attached in line with the vent ports of the mask. The pneumotachometer differential pressure was measured and converted to a voltage signal using a spirometer (ML141, ADInstruments) that delivered the voltage to the instrumentation amplifier.

Sleep stages were scored off-line according to the R and K criteria (Rechtschaffen and Kales 1968). An abrupt change in EEG frequency and/or brief increase in EMG amplitude for more than 3 s or over 15 s were classified as arousal or awake, respectively, as defined by the atlas task force of the American Sleep Disorder Association (AAoSMTF 1992). An arousal or wake was attributed to the odor stimuli if they occurred anywhere from odor onset to 30 s following odor offset (Stuck et al. 2007). Scoring for respiratory events, apnea and hypopnea, were according to the American Academy of Sleep Medicine (AAoSMTF 1999).

We tested for effects of odorants on 4 breath parameters: breath mean airflow velocity, breath maximum airflow velocity, breath volume, and breath duration. Breath volume was calculated by the trapezoidal Reimann sum method (Johnson et al. 2006). Breaths were aligned in time by setting the point at which the breath passed from the expiratory phase to the inspiratory phase as time 0.

Procedures

Subjects arrived at the olfaction sleep laboratory at a self-selected time, based on their usual sleep time, typically ~11:00 PM. After providing informed consent, subjects were led to the experimental room. This room was coated in stainless steel in order to prevent ambient odor adhesion and subserved by high-efficiency particulate air and carbon filtration to further assure an odor-free environment. Subjects first rated the intensity and pleasantness of the odorant using a visual analog scale (VAS). The VAS consisted of a line without any tick marks (14 cm long), with only the extremes marked as reflecting "very" or "not at all" (in Hebrew). After fitting of the polysomnography devices and assuring a comfortable positioning within the bed, subjects were left alone in the darkened room to be observed from the neighboring control room via IR video camera and 1-way observation window.

The experimenters observed the real-time polysomnography reading, and 20 min after they determined that the subject had entered stage 2 sleep, they initiated the experimental protocol that from this point on was computer controlled. Every 9, 12, or 15 min (randomized), the olfactometer generated a 5-, 10-, or 20-s (randomized) odor stimulus. This resulted in 21–37 odorant presentations per night. Upon spontaneous morning wake, subjects again rated the intensity and pleasantness of the odorant and were then debriefed, paid, and released.

Statistics

The obtained ratings for the respective odorant qualities were analyzed to test for differences between odorants in perception before and after the night. Odorant ratings were compared using a repeated measures analysis of variance (ANOVA) within Statistica software (StatSoft, Inc.). The ANOVA analysis was followed by contrast t-tests to test the difference between each 2 odorants. A P value of less than 0.05 was considered significant.

The number of arousals attributed to an odor divided by the total number of odor presentations was the "odor arousal frequency." The number of arousals attributed to an odorless baseline period (containing an equal flow of clean air) divided by the total number of odor presentations was the "baseline arousal frequency." Wake frequencies were calculated in the same manner. Frequencies were calculated for each subject and for each sleep stage. Frequencies of arousals and wakes were compared between and across sleep stages per odorant in a repeated measures ANOVA.

Averages of respiration measurements for inhalation, exhalation, and inhalation/exhalation ratio in the 30 breaths before odor onset were compared with the averages of respiration measurements for inhalation, exhalation, and inhalation/exhalation ratio for each one of 6 breaths after odor onset across sleep stages per odorant and for all odorants together. This analysis was corrected for the 6 comparisons using a Bonferroni correction. Respiratory measurements were compared between sleep stages per odorant in a repeated measures ANOVA.

Results

Psychophysical results

As intended, the odors differed in pleasantness [average across evening and morning: vanillin 9.0 ± 2.3, lavender oil 8.1 ± 2.1, ammonium sulfide 6.9 ± 1.2, and vetiver 4.6 ± 3.1; $F(3,30) = 6.34$, $P < 0.005$] (Figure 1). Lavender oil and vanillin were perceived as more pleasant than vetiver oil [all $F(1,14) > 7.09$, $P < 0.02$], and vanillin was perceived also as more pleasant than ammonium sulfide [$F(1,16) = 5.89$, $P < 0.03$]. There were no significant differences in odor intensities [averages
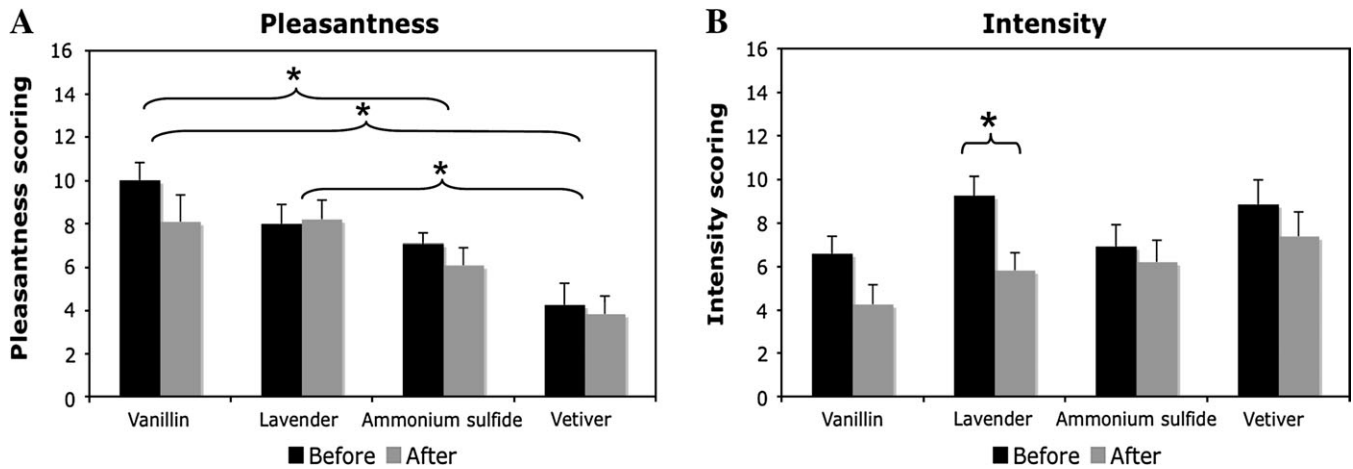
Figure 1 Pleasantness **(A)** and intensity **(B)** scoring from the evening (before) and the morning (after) for the 4 odorants (lavender oil, ammonium sulfide, vanillin, and vetiver oil). Error bars are standard error.

across evening and morning scores: vanillin 5.4 ± 2.2, lavender oil 7.5 ± 1.7, ammonium sulfide 6.6 ± 2.1, and vetiver 7.9 ± 2.8; $F_{(3,30)} = 2.4$, $P < 0.08$].

Odor pleasantness did not shift as a result of the nights' exposure for any odorant [evening scores: vanillin 10.0 ± 2.9, lavender oil 7.9 ± 2.5, ammonium sulfide 7.1 ± 1.2, and vetiver 4.3 ± 3.0; morning scores: vanillin 8.1 ± 4.3, ammonium sulfide 6.1 ± 2.0, lavender oil 8.2 ± 2.6, and vetiver 3.8 ± 2.5; all $F_{(1,11)} < 1.37$, $P > 0.2$]. In contrast, lavender oil intensity was perceived as weaker in the morning (5.8 ± 2.3) than in the evening [9.3 ± 2.5, $F_{(1,7)} = 9.11$, $P < 0.02$], and a similar trend was observed for vanillin [evening score: 6.6 ± 2.7, morning score: 4.25 ± 2.7; $F_{(1,11)} = 4.21$, $P < 0.06$]. There was no change in the intensities of ammonium sulfide and vetiver oil [evening scores: ammonium sulfide 6.9 ± 2.4 and vetiver oil 8.8 ± 3.4; morning scores: ammonium sulfide 6.2 ± 2.4 and vetiver oil 7.4 ± 3.4; all $F_{(1,7)} < 1.72$, $P > 0.3$].

Odorants did not arouse or wake

We initially tested the odorants vanillin ($n = 12$ after exclusions), lavender oil ($n = 13$ after exclusions), and ammonium sulfide ($n = 5$ after exclusions). For vanillin, there was no effect of odor on wake [$F_{(1,11)} = 2.23$, $P < 0.16$], regardless of sleep stage [$F_{(2,22)} = 0.009$, $P < 0.99$], and no effect of odor on arousal [$F_{(1,11)} = 2.93$, $P < 0.12$], regardless of sleep stage [$F_{(2,22)} = 1.98$, $P < 0.16$]. For lavender oil, there was no overall effect on arousal [$F_{(1,12)} = 0.13$, $P < 0.72$], with a sleep stage effect [$F_{(2,24)} = 8.68$, $P < 0.001$] reflecting increased arousal in stage 2 sleep compared with other sleep stages. There was a trend toward an effect on wakes [$F_{(1,12)} = 3.53$, $P = 0.084$], whereby lavender oil lowered the frequency of wakes, and a significant interaction between wake rates and sleep stage [$F_{(2,24)} = 3.37$, $P < 0.03$], reflecting increased wake in stage 2 sleep in the presence of odor compared with other sleep stages and baseline. For ammonium sulfide, there was no effect of odor on wake [$F_{(1,4)} = 1.0$, $P < 0.37$], regard-
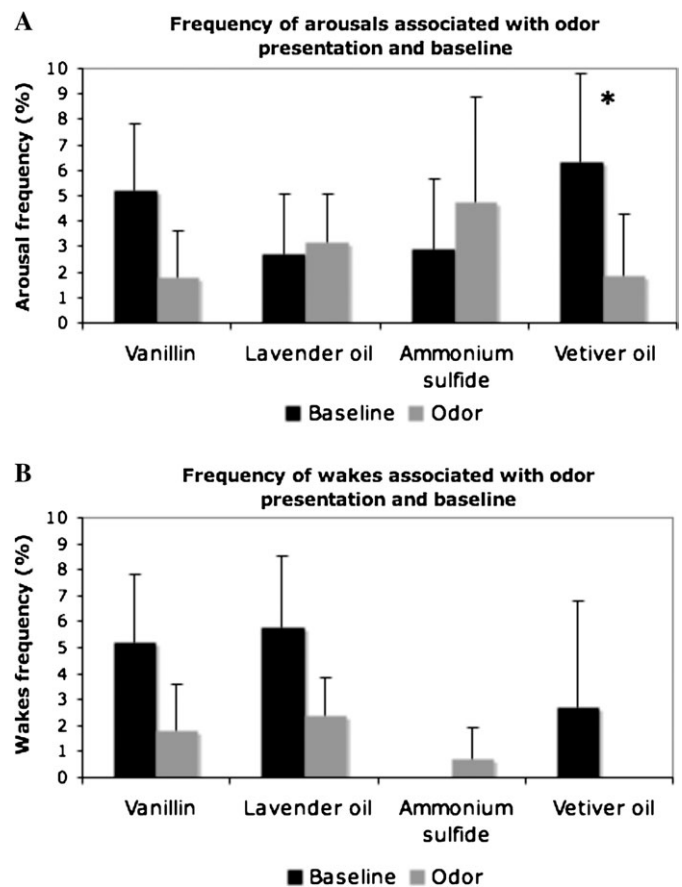

Figure 2 **(A)** Frequency of arousals associated with odor and baseline (clean air presented) in 4 odorants (lavender oil, ammonium sulfide, vanillin, and vetiver oil). **(B)** Frequency of wakes associated with odor and baseline (clean air presented) in 4 odorants (lavender oil, ammonium sulfide, vanillin, and vetiver oil). Error bars are standard error.

less of sleep stage [$F_{(2,8)} = 1.0$, $P < 0.41$], or arousal [$F_{(1,4)} = 0.56$, $P < 0.49$], regardless of sleep stage [$F_{(2,8)} = 0.45$, $P < 0.64$] (Figure 2, Table 1). Consistent with these results, ECG

Table 1 Wakes and arousal occurrence during odorant presentation and baseline

	Wakes frequency		Arousal frequency	
Odor/sleep stage	Vanillin baseline	Vanillin	Vanillin baseline	Vanillin
Stage 2	1.4 ± 2.5%	2.2 ± 5.2%	5.2 ± 8.9%	2.6 ± 4.8%
SWS	4.2 ± 14.4%	0 ± 0%	9.1 ± 7.8%	2.8 ± 9.6%
REM	3.8 ± 7.8%	0 ± 0%	4.4 ± 10.8%	0 ± 0%
All stages	3.1 ± 9.4%	0.7 ± 3.1%	10.4 ± 12.1%	1.8 ± 6.2%
Odor/sleep stage	Lavender oil baseline	Lavender oil	Lavender oil baseline	Lavender oil
Stage 2	3.8 ± 3.9%	7.0 ± 7.6%	4.0 ± 4.2%	9.5 ± 8.8%
SWS	6.9 ± 10.8%	0 ± 0%	4.2 ± 15.1%	0 ± 0%
REM	6.7 ± 13.5%	0 ± 0%	0 ± 0%	0 ± 0%
All stages	5.8 ± 9.8%	2.3 ± 5.4%	2.7 ± 8.4%	3.2 ± 6.7%
Odor/sleep stage	Ammonium sulfide baseline	Ammonium sulfide	Ammonium sulfide baseline	Ammonium sulfide
Stage 2	0 ± 0%	2.1 ± 4.7%	1.9 ± 2.6%	9.3 ± 10.9%
SWS	0 ± 0%	0 ± 0%	4.0 ± 8.9%	0 ± 0%
REM	0 ± 0%	0 ± 0%	2.9 ± 6.4%	5.0 ± 11.2%
All stages	0 ± 0%	0.7 ± 2.7%	2.9 ± 6.1%	4.8 ± 9.2%
Odor/sleep stage	Vetiver oil baseline	Vetiver oil	Vetiver oil baseline	Vetiver oil
Stage 2	0.8 ± 2.4%	0 ± 0%	7.3 ± 6.3%	1.4 ± 3.6%
SWS	0 ± 0%	0 ± 0%	6.2 ± 6.4%	0 ± 0%
REM	7.1 ± 18.9%	0 ± 0%	3.6 ± 9.4%	4.1 ± 10.8%
All stages	2.6 ± 10.9%	0 ± 0%	6.3 ± 9.2%	1.8 ± 6.4%

The percentage of odorant presentations accompanied by wakes or arousals by sleep stage and odorant. REM, rapid eye movement.

and blood oxygination measurements were not influenced by odor presentation [ECG levels at baseline = 57.96 ± 7.34 beats per minute (BPM), ECG levels after odor presentation = 58.04 ± 7.37 BPM; $F(1,26) = 0.23$, $P < 0.6$; blood oxygination at baseline 97.04 ± 1.22, blood oxygination after odor presentation 97.06 ± 1.23; $F(1,23) = 0.35$, $P < 0.56$]. The subjects' blood oxygination levels during wake were 97.66 ± 1.12% and total night average was 96.84 ± 1.56%.

Odors influenced respiratory patterns in sleep

The inhale/exhale volume ratio was significantly smaller following odor presentation in comparison to baseline for vanillin ($n = 12$ following exclusions), ammonium sulfide ($n = 5$ following exclusions), and lavender oil ($n = 13$ following exclusions) across all sleep stages (Figure 3, Table 2). This effect was most pronounced for the first breath following odorant onset [$F(1,35) = 384.51$, $P < 0.0001$] and then decreased in a nearly linear fashion until it was on the border of significance (Bonferroni corrected) at the sixth breath after odorant onset [$F(1,35) = 7.87$, $P < 0.0081$] (Figure 3). For all odorants, there was no difference in respiration volume ratio across sleep stages [vanillin $F(2,22) = 2.61$, $P < 0.1$;

lavender oil $F(2,24) = 3.00$, $P < 0.07$; ammonium sulfide $F(2,8) = 0.24$, $P < 0.79$].

We also examined breath inhalation and exhalation volume separately. Inhalation volume decreased significantly following odor onset for all 6 breaths after odor onset across the 3 odorants [all $F(1,29) > 14.2$, $P < 0.0007$]. Exhalation volume increased significantly in comparison to baseline for only the first breath after odor onset across the 3 odorants [$F(1,29) = 9.36$, $P < 0.005$] (Figure 4, Table 2).

The influence of odors on respiratory patterns in sleep reflected a temporary increase in net exhalation

The after odorant decrease in nasal inhalation and increase in nasal exhalation may have resulted from 2 alternative scenarios. In the first scenario, the odorant-induced temporary increase in the net nasal exhalation may have relied on the lung's air reserve. The second alternative is that, although oral respiration typically reflects only ~4% of overall respiration in sleep (Fitzpatrick et al. 2003), the odorant may have changed the balance between nasal and oral breathing such that oral inspiration increased. Under this scenario, the increase in nasal exhalation reflected an increase in oral
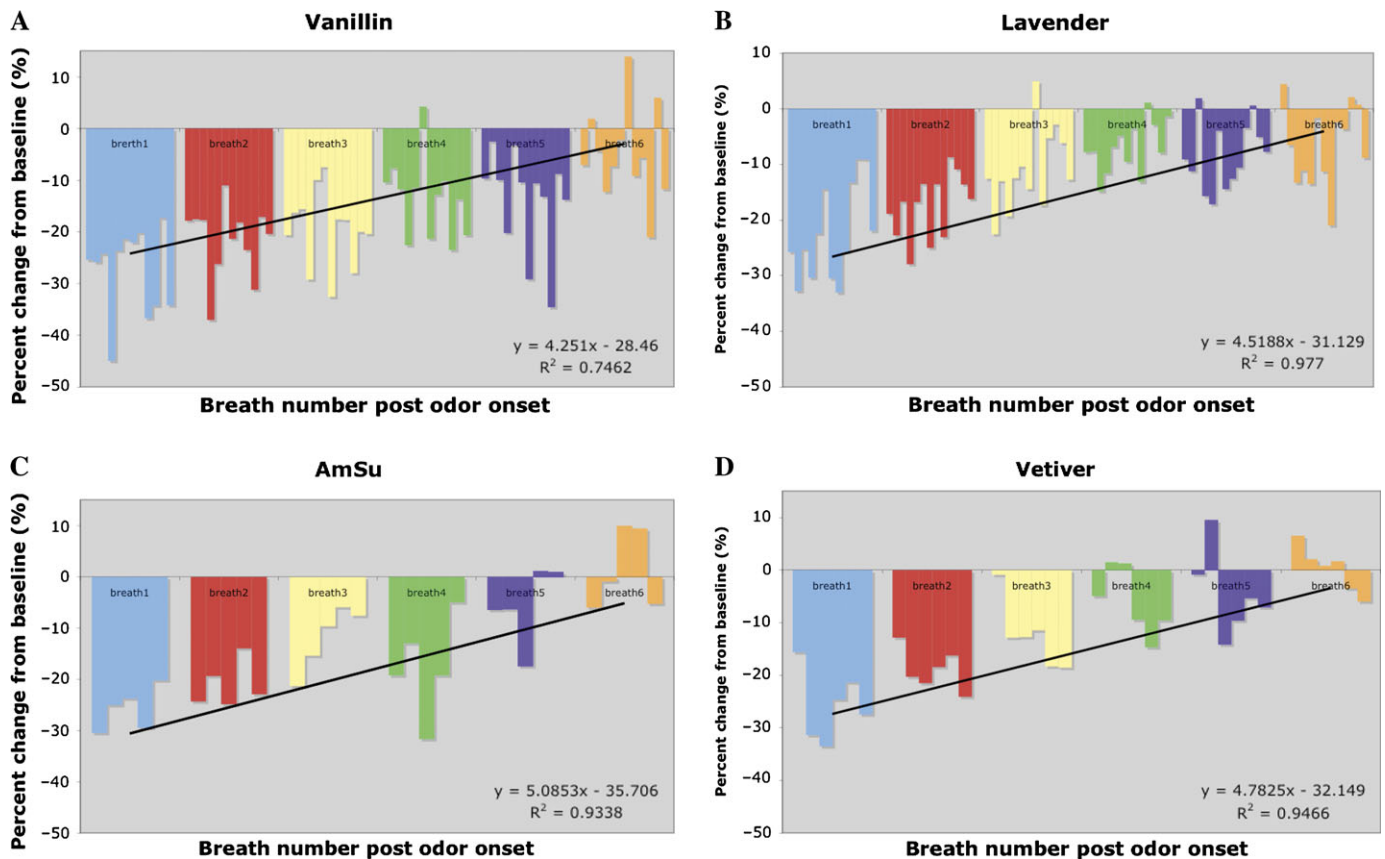
Figure 3 Percent change from baseline in the inhale/exhale volume ratio in 6 consecutive breathes following 1 of 4 odors: **(A)** ammonium sulfide, **(B)** vanillin, **(C)** lavender oil, and **(D)** vetiver oil. Each column represents the percent change from baseline in the inhale/exhale volume ratio for a single subject; each color represents a different breath after odor onset. The blank line is a linear trend line.

inspiration rather than the exhalation of air stored in the lungs. The above data could not discriminate between these alternatives because it relied on accurate measurement of nasal respiration alone. Therefore, we studied an additional control group of 10 subjects using the odorant vetiver oil that in addition to the nasal mask were fitted with an oral mask in order to accurately measure oral and nasal respiration simultaneously.

Regarding the effects of the odorant on sleep, the results with vetiver oil were similar to those with the 3 previous odorants. Seven subjects had full polysomnography data, and in these, there was a significant decrease in arousal frequency following odorant presentation [$F(1,6) = 28.13$, $P < 0.001$], regardless of sleep stage [$F(2,12) = 0.95$, $P < 0.41$], and no change in wake frequency [$F(1,6) = 1.29$, $P < 0.29$], regardless of sleep stage [$F(2,12) = 0.86$, $P < 0.45$] (Figure 2, Table 1).

Regarding the effects of the odorant on respiration, only 0–18% of overall after odorant respiration was oral, and it was indeed nasal respiration that carried the previously observed effects, whereby the odorant reduced inhale/exhale volume ratio across all sleep stages (Figure 3, Table 2) (1 subject had a stuffed nose and was therefore excluded from this analysis). In analyzing the nasal respirations, this effect was sig-

nificant for 3 consecutive breathes following odor onset [all $F(1,5) > 22.34$, $P < 0.006$], regardless of sleep stage [$F(2,10) = 1.127$, $P < 0.36$]. In all 4 odorants, there was a ~30% change in inhale/exhale volume ratio in the first breath following odor onset (Figure 5). Similar to the other odorants, the effects in vetiver oil were a result of a decrease in inhalation and increase in exhalation volume that remained significant for 2 breaths following odor onset [all $F(1,5) > 11.02$, $P < 0.02$]. In other words, the change that was found in respiration pattern during sleep reflected a temporary increase in net nasal exhalation that relied on the lung's air reserve.

Although all odorants tested had a similar type of influence on respiration, we combined the results using vetiver oil with those using ammonium sulfide in order to allow a better test for any influence of odorant valence on respiration in sleep. This combination generated 1 group of 11 subjects tested with unpleasant odorants (vetiver oil and ammonium sulfide) and 2 separate groups of 12 (vanillin) and 13 (lavender oil) subjects tested with pleasant odorants. We found no difference in the effect size of the inhale/exhale volume ratio across the odorants [all $F(2,33) < 2.23$, $P > 0.12$], and follow-up tests revealed no significant differences in either inhale or exhale change across valences [all $F(2,33) < 1.37$, all $P > 0.2$].

Table 2 Respiration volume during odor presentation and baseline

Breath/odor	Baseline	Breath 1	Breath 2	Breath 3	Breath 4	Breath 5	Breath 6
Inhale/exhale volume ratio							
Vanillin	1.00	0.73 ± 0.08***	0.78 ± 0.07***	0.80 ± 0.07***	0.87 ± 0.08***	0.86 ± 0.1***	0.95 ± 0.09
Lavender oil	1.00	0.77 ± 0.09***	0.83 ± 0.06***	0.90 ± 0.07***	0.93 ± 0.05***	0.92 ± 0.06***	0.93 ± 0.07*
Ammonium sulfide	1.00	0.74 ± 0.04***	0.80 ± 0.05***	0.88 ± 0.06*	0.82 ± 0.09*	0.94 ± 0.08	1.01 ± 0.08
Vetiver oil	1.00	0.74 ± 0.07***	0.81 ± 0.04***	0.87 ± 0.06*	0.94 ± 0.06	0.95 ± 0.08	1.00 ± 0.04
All odorants	1.00	0.75 ± 0.08***	0.80 ± 0.06***	0.86 ± 0.08***	0.89 ± 0.08***	0.91 ± 0.08***	0.96 ± 0.08**
Inhalation volume (ml)							
Vanillin	164.63 ± 62.96	126.33 ± 55.64***	132.54 ± 56.78***	136.05 ± 57.79***	140.76 ± 60.06***	142.77 ± 61.94***	146.49 ± 58.67*
Lavender oil	138.21 ± 47.25	116.39 ± 48.86***	119.36 ± 50.62***	123.56 ± 48.52***	130.79 ± 50.92	129.96 ± 46.65**	129.85 ± 49.85*
Ammonium sulfide	229.24 ± 93.92	193.40 ± 94.90*	196.22 ± 87.06	212.98 ± 92.53	195.75 ± 101.68	212.17 ± 94.47	217.90 ± 89.61
Vetiver oil	209.94 ± 84.00	185.48 ± 84.09*	194.63 ± 82.12*	202.08 ± 81.76	210.94 ± 81.50	215.34 ± 85.44	216.52 ± 84.80
All odorants	171.61 ± 72.01	141.92 ± 69.72***	146.98 ± 69.36***	153.23 ± 71.67***	156.50 ± 72.19***	159.88 ± 73.24***	162.07 ± 72.73**
Exhalation volume (ml)							
Vanillin	164.63 ± 62.96	174.21 ± 71.71	167.87 ± 68.48	165.68 ± 64.72	160.11 ± 63.54	161.28 ± 62.38	155.94 ± 155.94
Lavender oil	138.21 ± 47.25	147.54 ± 54.66*	143.29 ± 56.01	138.84 ± 51.90	139.79 ± 50.94	140.84 ± 47.41	137.09 ± 45.72
Ammonium sulfide	229.24 ± 93.93	256.93 ± 117.13	246.67 ± 107.19	238.39 ± 97.41	230.76 ± 99.54	222.07 ± 89.59	215.84 ± 95.11
Vetiver oil	209.94 ± 84.01	245.06 ± 104.99*	239.31 ± 101.09*	233.06 ± 100.94	229.69 ± 99.14	226.24 ± 93.14	217.34 ± 89.00
All odorants	171.62 ± 72.01	187.87 ± 87.71***	181.85 ± 84.30*	177.32 ± 80.26	174.18 ± 78.67	173.17 ± 73.56	167.69 ± 72.78

Averages of inhale/exhale volume ratio, inhalation, and exhalation volume in 6 breaths after odor onset and baseline. Significance level of the comparison between baseline and after odor breath: *$P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.
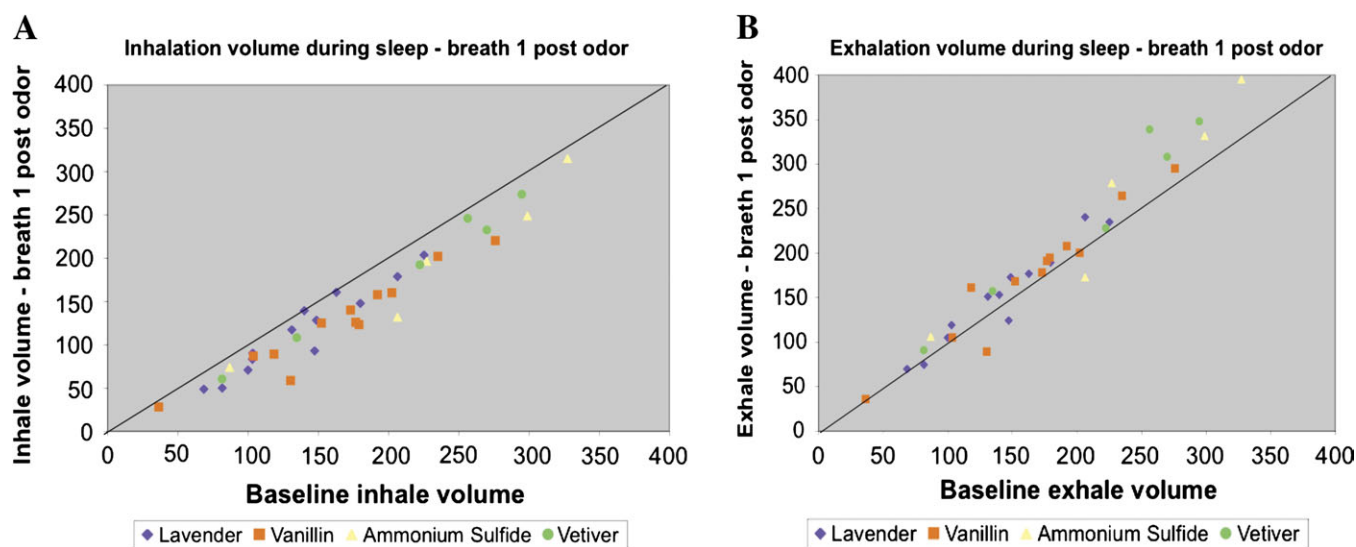

Figure 4 Breath inhale **(A)** and exhale **(B)** volume in the first breath following odor onset (Y axis) and baseline without odor (X axis). The diagonal line is the unit slope line. Thus, if points accumulate above the line, the parameter was greater following odor. If under the line, then the parameter was greater at baseline (reduced by odor). If the points are around the line, the parameter was unaffected by odor. The units in the graphs are in mL.

To further examine the influence of odor valence on respiratory volume ratio, we directly compared between pleasant odorants (vanillin and lavender oil) and the unpleasant odorants (vetiver oil and ammonium sulfide). We found that the inhale/exhale volume ratio was not influenced by valence [all $F(1,32) < 5.59$, $P =$ not significant following correction].
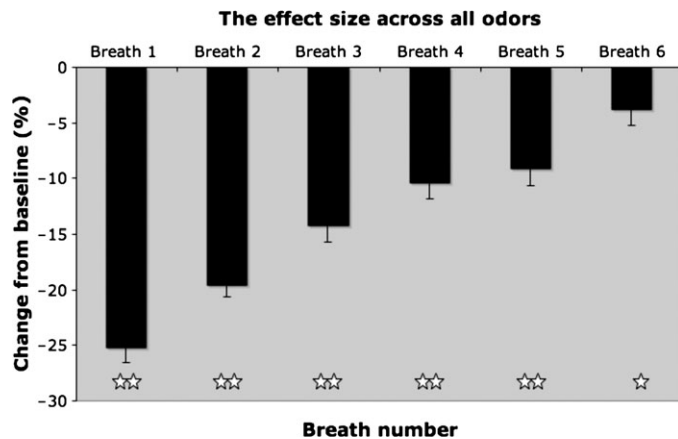
The effect size across all odors

Figure 5 Percent change from baseline of the inhale/exhale volume ratio for 6 consecutive breathes following odor onset. The effect was significant for 6 consecutive breathes following odor onset across all 4 odorants. Error bars are standard error. ll, $P < 0.001$; l, $P < 0.0083$.

Moreover, to further examine the influence of trigeminality on respiratory volume ratio, we directly compared between mildly trigeminal odorants (lavender oil and vetiver oil) and pure olfactants (vanillin and ammonium sulfide). We found that the inhale/exhale volume ratio was greater for pure olfactants only at the fourth breath after odorant onset [pure olfactants = 0.15 ± 0.08, mild trigeminal odors = 0.07 ± 0.05; $F(1,32) = 13.1$, $P < 0.001$].

Finally, the entire above analysis depicted the results considering the measure of breath volume. Consistent with previous studies (Youngentob et al. 1987), breath volume was correlated to the 3 other breath measures (volume and duration, $r = 0.73$, $P < 0.0001$; volume and maximum airflow velocity, $r = 0.57$, $P < 0.07$; volume and mean airflow velocity, $r = 0.56$, $P < 0.03$). Repeating the analysis with either of these respiratory measures revealed a nearly identical picture.

Discussion

Consistent with previous studies, we found that odors did not arouse or wake (Badia et al. 1990; Carskadon and Herz 2004; Stuck et al. 2007; Grupp et al. 2008). In fact, to the extent that odorants had any influence on arousal and wake, they reduced both. In other words, odorants may potentially improve the quality of sleep. This too is consistent with previous findings. For example, presentation of peppermint for ~40 min before sleep increased total sleep time and proportion of slow-wave sleep (SWS) in those subjects who found peppermint very intense (Goel and Lao 2006). In a similar study, lavender oil significantly increased SWS compared with an odorless control (Goel et al. 2005). In addition, lavender, but not almond oil, improved sleep quality in people with mild insomnia (Lewith et al. 2005), and infants given a bath with lavender-scented bath oil later spent more time

in deep sleep (Field et al. 2008). Our findings of reduced arousal and wake frequency following presentations of lavender and vetiver oil were consistent with the above studies.

In turn, the novel finding we report here is that odors modified respiration during sleep, where they decreased inhalation and increased exhalation volume for several breaths after odor onset. In other words, there was a temporary respiratory rejection type response. This effect persisted regardless of odorant valence and was consistent for both pure olfactants and mild trigeminals (accept 1 time point in 1 analysis where the effect was greater for the pure olfactants).

In wake, sniffs are larger for pleasant than for unpleasant odorants (Bensafi et al. 2007). Here we found that odorant-induced alterations in respiration were valance independent. This implies a possible difference in the responsiveness of the olfactory system between sleep and wake. Several lines of evidences support the notion that odors are processed in sleep. In humans, odor presentation in sleep influenced cognitive functions such as memory (Rasch et al. 2007), modified EEG, increased heart rate, and reduced EMG (Badia et al. 1990). Similarly, odors reduced locomotor activities and body movements during sleep in rats (Sano et al. 1998). These findings indicate that the olfactory system does process odors during sleep, but they do not tell us if it processes them as in wake. In turn, direct in vivo recordings from primary olfactory cortex in rats suggested an altered responsiveness in sleep: neurons in the olfactory cortex enter different modes of membrane potential fluctuations (slow- and fast-wave states) during different behavioral states. In the slow-wave state, which could be compared with sleep, there was reduced responsiveness to odor stimuli compared with the fast-wave state, which more resembles wake (Murakami et al. 2005). However, even during slow-wave activity, slow oscillations in the olfactory system were highly correlated with the rhythmic entry of odorless air into the nostrils during baseline respiration (Fontanini and Bower 2006), suggesting that the olfactory cortex is tuned to process sensory stimuli during slow-wave activity as well. To conclude on this front, the current data add an example of olfactory processing during sleep that differs from the processing in wake. However, it does not allow us to disambiguate weather the loss of odor hedonic-specific respiratory responses reflected a sleep-related difference in the processing of the olfactory stimulus within the olfactory system or a sleep-related difference in coupling of the olfactory system to the respiratory response.

A number of studies have examined the influence of odors on respiration in wake (Warren et al. 1994; Sobel et al. 1999; Walker et al. 2001; Johnson et al. 2003; Bensafi et al. 2007), but only 1 study addressed this issue in healthy adults during sleep. In contrast to our findings, Badia et al. (1990) found that 3 min of peppermint presentation did not change respiration in comparison to nonfragrance periods. However, respiration was not the focus of Badia et al. (1990),

and this was reflected in several methodological aspects that may explain the discrepancy between their results and ours here. First, here we measured respiration breath-by-breath and found that the influence of odorants on respiration in sleep typically persisted for 6 breaths after odorant onset (equivalent to ~30 s). Badia et al. (1990) binned respiration into 3-min epochs, likely obscuring changes that occurred on the timescale observed here. Second, whereas here we measured respiration with highly sensitive pneumatotacho-graphs, Badia et al. (1990) used respiratory belts to measure respiration. Although such belts provide a good measure of overall respiration, they lack the sensitivity to reveal the dif-ferences in respiration typically induced by odorants (Johnson et al. 2006). Taken together, we think that these methodological differences account for the difference in re-sults across studies. A second study addressed the influence of odor on sleep respiration in premature newborns with apnea (Marlier et al. 2005). In this study, 15 drops of van-illin were applied to the periphery of the infant's pillow every 12 h across 1 day. Heart rate and respiration were re-corded using a clinical monitor (Viridia), and oxygen saturation was measured using a pulse oximeter. The odor diminished apnea occurrence by 36% (Marlier et al. 2005). In a third study, Seelke and Blumberg (2004) presented sleeping and awake infant rats with cotton-tipped applica-tors dipped in the dimethyl disulfide (DMDS) solution at 1 of 4 concentrations or saline. Respiration was monitored with a plethysmograph in both sleep and wake. They found no effects of DMDS concentration on arousal, but in con-trast, they found that DMDS changed respiration by elic-iting short-lasting polypnea. Although the mechanisms of apnea may differ in premature newborns and adults and be-tween rats and humans, the results of Marlier et al. (2005) and Seelke and Blumberg (2004) combine with ours here to suggest that odors are a viable instrument for modifying respiratory patterns in sleep.

In conclusion, we set out to ask 2 simple questions: 1) do odorants transiently presented during sleep induce arousal or wake? and 2) do odorants transiently presented during sleep induce a respiratory response? Regarding the first ques-tion, in agreement with previous findings, our results sug-gested that mildly trigeminal and pure olfactory odorants do not arouse or wake, and to the extent that they influence arousals and wakes at all, they in fact reduce them. Regard-ing the second question, we found that odorants transiently presented during sleep induced a respiratory rejection type response, whereby net exhalation was increased for up to 6 breathes after odorant onset. This finding suggests that our intent of manipulating the respiratory system without waking is viable. However, considering the dynamics of ap-nea, one may question whether such respiratory manipula-tion as evidenced here will have any influence on apnic events. This, however, can only be answered by now replicat-ing the current study in patients with apnea, and we think that the current results merit such a test.

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