

Increasing Humidity Blocks Continuous Positive Airflow-induced Apnea Responses in Rats

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Background: The aim of this study was to investigate the role of temperature and humidity in positive continuous pressure flow-induced apnea.

Methods: Forty-two adult male Sprague-Dawley rats were used in a functionally isolated laryngeal animal model. In study 1, animals were challenged with laryngeal stimulation for 1 minute to detect the possibility of adaptation. In study 2, different airflow conditions (25°C dry–25°C dry, 25°C dry–25°C wet, 25°C dry–37°C dry and 25°C dry–37°C wet) were delivered to determine the role of temperature and humidity in the flow-induced apneic response of the larynx. The apneic index was calculated by prolonged expiratory time/baseline expiratory time.

Results: Laryngeal dry room temperature air exposure induced an apneic response, and this response was reproducible and could be eliminated by humidification. In contrast, this apneic response could not be inhibited by increasing temperature alone. In addition, prolonged cold dry air stimulation did not evoke a tachyphylactic effect to normalize the breathing pattern.

Conclusion: Laryngeal cold dry air stimulation triggered an apneic response, which could be eliminated by humidification but not by the heating of air. These results suggest that using continuous positive airway pressure (CPAP) with humidified air decreases CPAP-induced apnea. [*J Chin Med Assoc* 2010;73(7):369–374]

Key Words: constant air flow, continuous positive airway pressure, humidification, obstructive sleep apnea, respiratory reflex

Introduction

Brief periods of breathing cessation (apnea) or a marked reduction in tidal volume (hypopnea) are common in adults during sleep.¹ Sleep apnea is estimated to affect 4–25% of the adult population.² The most common form of sleep apnea is obstructive sleep apnea (OSA). It is characterized by repetitive episodes of complete or partial upper airway obstruction leading to absent or diminished airflow into the lungs with persistence of ventilatory effort, as shown by paradoxical chest and abdominal movement, and varying degrees of oxygen desaturation.^{3,4} Nasal continuous positive airway pressure (CPAP) devices have remained the mainstay of treatment of patients with OSA since they were first introduced in 1981.^{2,5} CPAP is the application of

positive pressure to the airways of the spontaneously breathing patient throughout the respiratory cycle.^{6,7} In randomized, placebo-controlled trials, CPAP has been shown to decrease somnolence and to improve quality of life, mood, and alertness.^{8,9} However, high pressure airflow through the upper airway has an inhibitory effect on respiration in animals and humans.^{10,11} How to improve this adverse effect of CPAP is an important issue in the treatment of OSAs.

Using CPAP with heated humidity results in a reduction of the side effects associated with upper airway symptoms,¹² but it is not clear whether the change in temperature and humidity might influence the respiratory responses to CPAP. Although it has been found that cooling of the upper airway, which stimulates specific cold receptors and inhibits laryngeal



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mechanoreceptors, reduces respiratory activity in unanesthetized humans and anesthetized animals,¹³ airflow in these experiments resulted in uncertainty as to whether reflex apnea was induced by the change in temperature or humidity. Therefore, the role of temperature and humidity in CPAP-induced apnea needs further clarification.

In this study, we investigated the role of temperature and humidity in continuous positive pressure flow-induced apnea using a functionally isolated laryngeal animal model.

Methods

General preparations

Adult male Sprague-Dawley (SD) rats were anesthetized with an intraperitoneal injection of α -chloralose (100 mg/kg; Sigma, St Louis, MO, USA) and urethane (500 mg/kg; Sigma). The femoral artery and jugular vein were cannulated for recording arterial blood pressure and for the administration of pharmacological agents, respectively. During the experiment, the depth of anesthesia was regularly monitored at fixed intervals. Supplemental doses of α -chloralose (20 mg/kg/hr) and urethane (100 mg/kg/hr) were administered intravenously whenever necessary to maintain abolition of the pain reflex induced by pinching the animal's tail. The animals were tethered in a supine position, the neck opened in the midline, and the esophagus ligated as rostrally as possible. The trachea was cannulated below the larynx with a short tracheal cannula via a tracheostomy, throughout which the animals were breathing spontaneously. It took approximately 1 hour to complete the animal preparation. Body temperature was maintained at 36°C throughout the experiment by means of a servo-heating blanket. All protocols were in accordance with the guidelines for the care and use of laboratory animals published by the National Institutes of Health (Bethesda, MD, USA), and were approved by the Committee of the Taipei Veterans General Hospital, Taiwan (VGH 97-139).

Functionally isolated laryngeal preparation

The isolated laryngeal preparation was carried out as previously described.¹⁴ Briefly, after the trachea was exposed, 2 short tracheal cannulae were inserted via tracheostomy. One (PE-90) was inserted caudally just above the thoracic inlet, and the other (PE-60) cranially, with its tip placed slightly below the cricoid cartilage. A latex cuffed tube (Mallinckrodt 86442; 3 mm internal diameter) was introduced through the mouth with its tip placed at the pharynx. The position of this

oral tube was then fixed to the upper jaw of the rat, the cuff was inflated, and small cotton balls stuffed in the oral and nasal cavities to prevent any air leakage. During the experiment, rats breathed spontaneously via the lower tracheal cannula. The respiratory flow and tidal volume were recorded and restored.

Experimental procedures

In 42 adult male SD rats, different levels of airflow (50, 100, 200, 300 and 400 mL/min for 15 seconds) were administered into a functionally isolated larynx to detect the threshold for inducing reflex apnea. In study 1, which used 10 rats, a lower airflow that could evoke apnea was used for a long stimulation (1 minute) to detect the possibility of adaptation. In study 2, which used 32 rats, the animals were equally divided into 4 groups: a 25°C dry air challenge followed by a 25°C dry air challenge (25°C dry–25°C dry), a 25°C dry air challenge followed by a 25°C humidified air challenge (25°C dry–25°C wet), a 25°C dry air challenge followed by a 37°C dry air challenge (25°C dry–37°C dry), and a 25°C dry air challenge followed by a 37°C humidified air challenge (25°C dry–37°C wet). In the laryngeal application tests, 30 minutes elapsed before the next challenge.

Data and statistical analysis

Expiratory duration (T_E), respiratory frequency (f), tidal volume (V_T), and respiratory flow (V) were analyzed on a breath-by-breath basis and were averaged into 1-second intervals. The respiratory parameters were measured at least 10 breaths before and 20 breaths after airflow challenge. Baseline data for each respiratory parameter were calculated as the mean over 10 breaths immediately before airflow delivery. Mean arterial blood pressure was measured at 1-second intervals. These physiological parameters were analyzed using a computer equipped with an analog-to-digital converter (DASA 4600; Gould Instrument Systems Inc., Valley View, OH, USA) and software (BioCybernetics 1.0; Taipei, Taiwan). Results obtained from the computer analysis were routinely checked with those obtained by manual calculation for accuracy. Within-group comparisons were evaluated by a paired t test. A p value < 0.05 was considered to be significant. All data are presented as mean \pm standard error.

Results

The threshold of induced apneic responses was individually different. The lower airflow that could evoke apnea was used for a long stimulation (1 minute) to

detect a tachyphylactic reaction. A 25°C dry airflow through the isolated larynx induced an apneic response in spontaneously breathing SD rats, and this response was reproducible (Figures 1A, 1B and 4A). The apnea indices of the first and second 25°C dry air stimulation were 1.98 ± 0.11 and 1.90 ± 0.08 , respectively (Figure 3A), which were not significantly different ($p=0.54$). This respiratory inhibitory reaction persisted during the long stimulation period. Therefore, prolonged stimulation did not evoke tachyphylaxis in our animal model.

When stimulatory air was saturated with water, the cold dry air-induced apneic response was totally eliminated (Figures 1C, 1D, 2C, 2D and 4B). In the 25°C dry–25°C wet group, the apnea index of 25°C dry air (2.01 ± 0.20) was significantly higher than that of 25°C wet air (1.02 ± 0.03 , $p=0.002$) stimulation (Figure 3B). In the 25°C dry–37°C wet group, the apnea index of 25°C dry air (2.03 ± 0.146) was significantly higher than that of 37°C wet air (1.01 ± 0.03 , $p=0.001$) stimulation (Figure 3D). These results indicated that

humidity could improve cold dry air-induced apnea, regardless of what the temperature was.

Only increasing the temperature of stimulatory air could not inhibit the apneic response (Figures 2A and 2B). The apnea indices of 25°C and 37°C dry air stimulation were not significantly different (2.07 ± 0.11 vs. 2.13 ± 0.22 , $p=0.79$; Figure 3C). These results demonstrated that temperature played a minor role in cold dry air-induced apnea.

Discussion

We showed that cold dry air laryngeal stimulation induced an apneic response in SD rats. This response was totally eliminated by humidification of the air. However, it was not affected by the heating of air. This result demonstrates that humidification of air plays an important role in air-induced apnea in the larynx and the temperature of air does not change this effect.

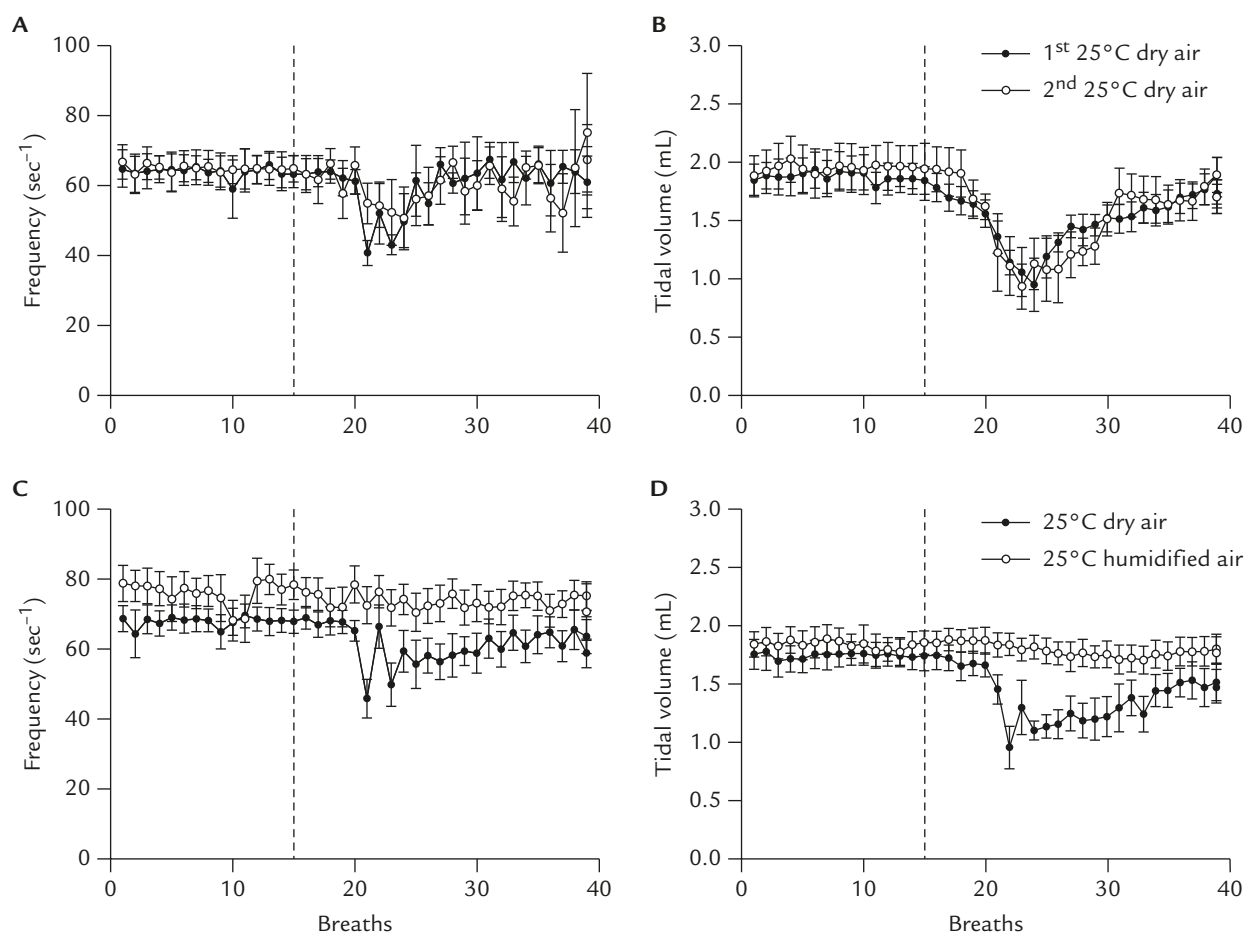


Figure 1. Mean ventilatory responses evoked by laryngeal exposure to 25°C dry air and 25°C humidified air in 16 animals. (A, B) Animals ($n=8$) responded to a 25°C dry air challenge followed by a 25°C dry air challenge. (C, D) Animals ($n=8$) responded to a 25°C dry air challenge followed by a 25°C humidified air challenge. The vertical dotted line indicates the start of air flow delivered into the isolated larynx. Data are mean \pm standard error.

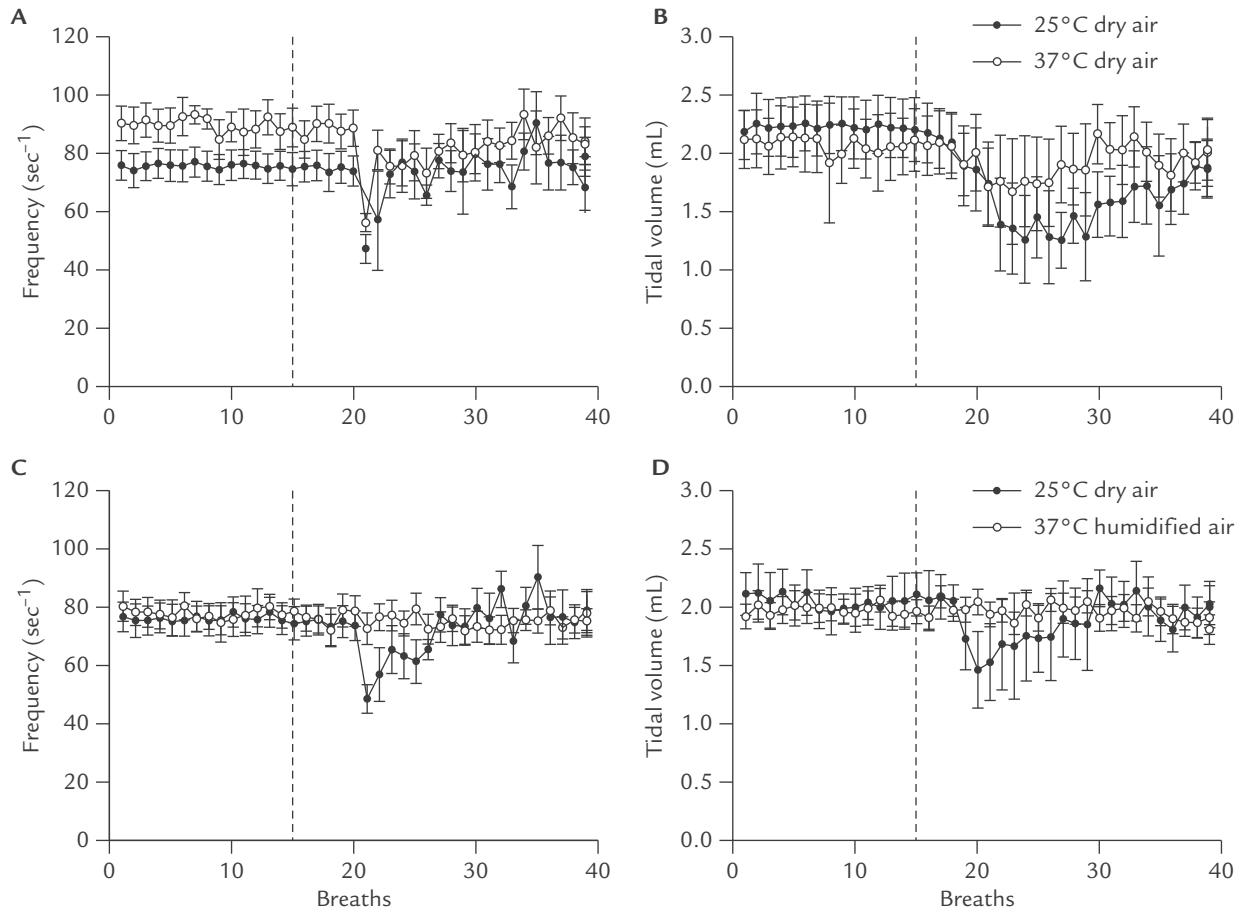


Figure 2. Mean ventilatory responses evoked by laryngeal exposure to 37°C dry air and 37°C humidified air in 16 animals. (A, B) Animals ($n=8$) responded to a 25°C dry air challenge followed by a 37°C dry air challenge. (C, D) Animals ($n=8$) responded to a 25°C dry air challenge followed by a 37°C humidified air challenge. The vertical dotted line indicates the start of air flow delivered into the isolated larynx. Data are mean \pm standard error.

In addition, prolonged cold dry air stimulation did not evoke the tachyphylactic effect to normalize the breathing pattern. This indicates that a protective effect cannot be evoked by prolongation of cold dry air laryngeal stimulation in SD rats.

Despite the high prevalence of OSA syndrome, no ideal therapy has emerged. CPAP devices are the mainstay of treatment for patients with OSA.^{2,5} In 1977, Lee et al showed that stimulation of the laryngeal afferents innervating the laryngeal mucosa could induce central apnea.¹⁵ Furthermore, prolonged laryngeal reflex stimulation results in asphyxial death in animal models.^{15,16} In 2005, Sun and colleagues found that the inhibition of phrenic nerve activity is continuous during the period of laryngeal hyperventilatory stimulation.¹⁷ Our results also showed that cold dry air through the isolated larynx induced an apneic response in anesthetized spontaneously breathing rats. Prolonged cold dry air laryngeal stimulation (for 1 minute) did not evoke a tachyphylactic effect to normalize the breathing

pattern. This result suggests that treatment with CPAP can induce an apneic reaction in OSA patients. This response may decrease the compliance of the patients, even inducing more severe complications.

In 1985, investigators using a single nerve recording demonstrated that the discharge rate of the laryngeal receptors was inversely related to laryngeal temperature.¹⁸ All the receptors were suppressed when laryngeal temperature was raised to 35–38°C with 100% relative humidity, and increased their activity when the laryngeal temperature was decreased. Previous studies have demonstrated that cool air can cause a decrease in respiratory frequency and peak inspiratory flow when applied to the upper airway in anesthetized rats.¹⁹ They also found that this effect may be of a reflex nature from the upper airway and laryngeal cold receptors could be mediated with this response. Adding heat to the respiratory tract by actively warming air before inhalation can potentially decrease resting bronchial tone and produce symptomatic improvement in asthmatic

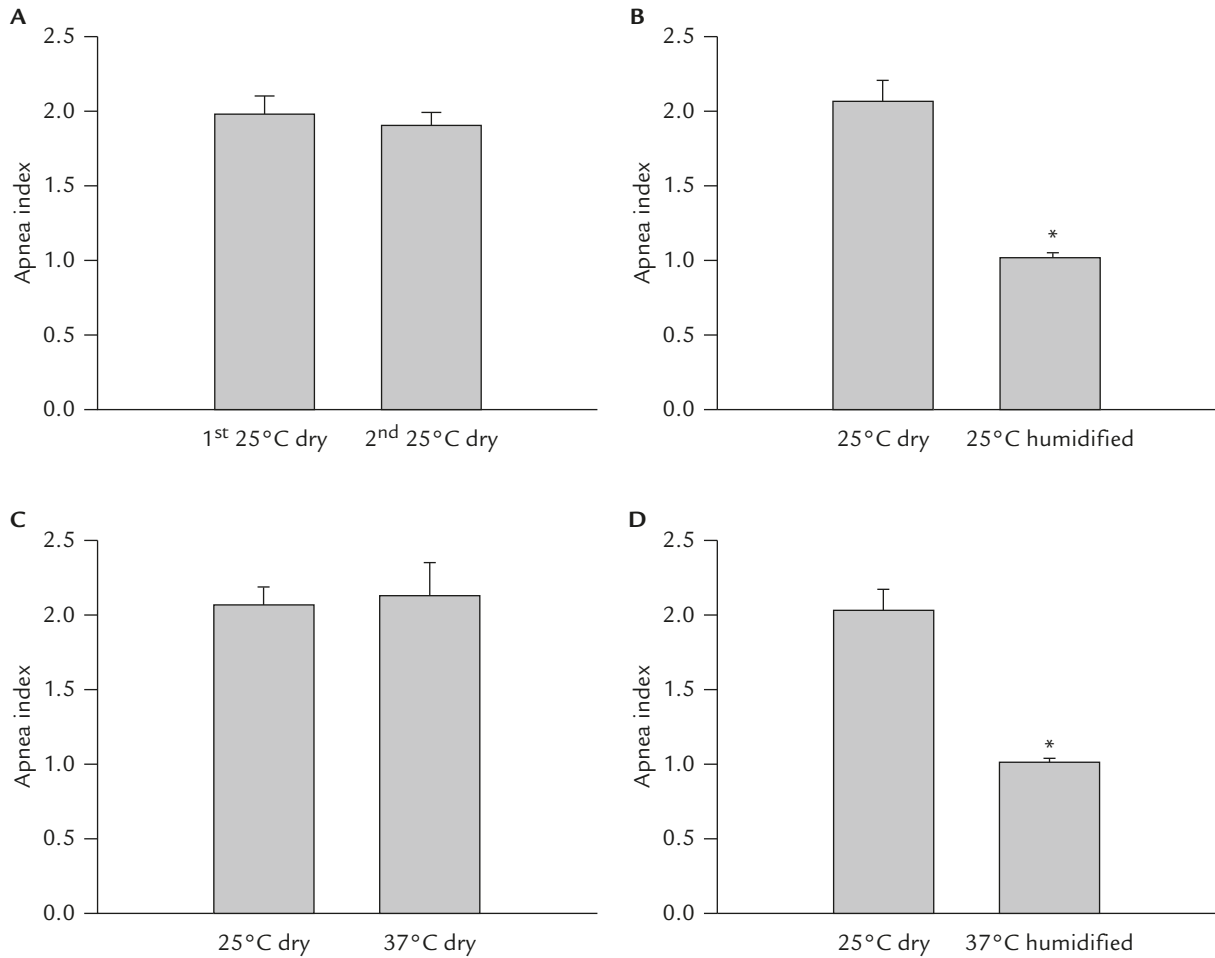


Figure 3. Mean apneic responses evoked by laryngeal exposure to different conditions of air flow. (A) A 25°C dry air challenge followed by a 25°C dry air challenge. (B) A 25°C dry air challenge followed by a 25°C humidified air challenge. (C) A 25°C dry air challenge followed by a 37°C dry air challenge. (D) A 25°C dry air challenge followed by a 37°C humidified air challenge. * $p < 0.05$ vs. control response. Data are mean \pm standard error.

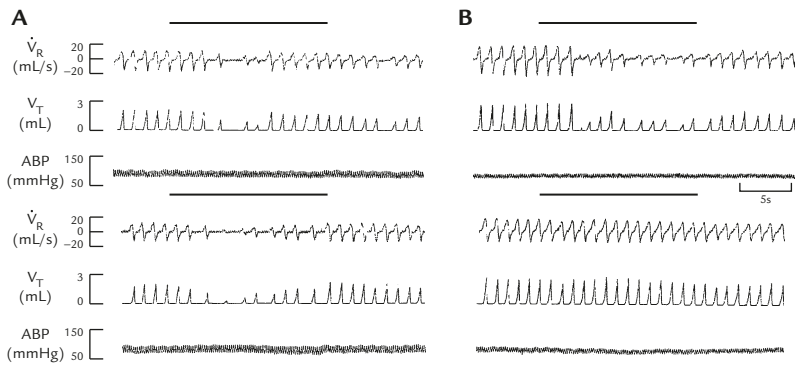


Figure 4. Experimental records illustrating immediate responses evoked by a laryngeal challenge with different conditions of air flow. (A) A 25°C dry air challenge followed by a 25°C dry air challenge. (B) A 25°C dry air challenge followed by a 25°C humidified air challenge. The horizontal bar at the top of all traces indicates the duration of the airflow stimulation. The time between the 2 challenges was 30 minutes. V_R =respiratory flow; V_T =tidal volume; ABP=arterial blood pressure.

patients.²⁰ Our results showed that cold dry air laryngeal stimulation induced an apneic response, which was totally eliminated by humidifying the air, but it was not affected by only heating of the air. This finding suggests that the humidity of inspiratory air plays a more important role than temperature in cold dry air-induced

apnea. We demonstrated that using CPAP with humidified air decreased CPAP-induced apnea and increased the compliance of using CPAP in OSA patients.

In conclusion, we found that continuous cold dry air stimulated the larynx, which induced an apneic response in SD rats. This response could be eliminated

by increasing the humidity. This finding indicates that using CPAP with humidified air can decrease CPAP-induced apnea and increase the compliance of using CPAP in OSA patients.

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