Mild Hypoxia Does Not Suppress Auditory Arousal From NREM Sleep

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Study Objectives: The depressive effects of hypoxia on the central nervous system are well known. The purpose of this study was to determine the influence of mild overnight hypoxia on the ability of healthy individuals to arouse from non-rapid-eye-movement (NREM) sleep to auditory tones.

Design: Randomized cross-over.

Setting: Participants slept in a sound-insulated room with the physiologic recordings and experimental interventions controlled from a separate room.

Participants: Eleven healthy men aged 18 to 24 years.

Interventions: On separate nights, participants were exposed to mild overnight hypoxia (SaO2 ~80%) or medical air in single-blind fashion. During established sleep, subjects were administered 1 of 10 auditory tones (500 Hz, 54-90 dB, 5 seconds duration) via earphones, or a sham tone (recording period with no tone).

Measurements and Results: The probability and intensity of arousal responses in the 30 seconds following tones or shams were compared between gas conditions and between stage 2 and slow-wave sleep. Arousal probability and intensity increased with tone intensity and were significantly lower during slow-wave compared with stage 2 sleep but were not different between hypoxia and normoxia nights.

Conclusion: These data suggest that mild overnight hypoxia does not impair the neural mechanisms involved in arousal from sleep to auditory stimuli.

Keywords: Arousal threshold, hypoxia, sleep, audition, arousal

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INTRODUCTION

AROUSAL FROM SLEEP IS CONSIDERED ONE OF THE MOST IMPORTANT PROTECTIVE RESPONSES TO ADVERSE RESPIRATORY EVENTS SUCH AS OBSTRUCTIVE apnoea.1 Arousal produces intense cardiovascular and respiratory activation that rapidly reverses blood-gas disturbance associated with airway obstruction and likely promotes physiologic preparedness for subsequent “fight-or-flight” survival responses. In healthy individuals, airway obstruction invariably precipitates rapid arousal.2 Patients with obstructive sleep apnea (OSA) show less-consistent and delayed arousal consistent with an increased arousal threshold to respiratory stimuli.3 While some obstructive events appear to resolve without arousal, and airway reopening precedes arousal in approximately 20% of events, arousal is nonetheless elicited in the majority of events in OSA.4

Hypoxia is an invariably accompanying component of sleep-disordered breathing. Experimental acute hypoxia (e.g., 0.5 to 2-3 minutes) during sleep induces a rapid ventilatory response followed by arousal.5 In contrast, indirect evidence supports that, following more-sustained hypoxia or repeated brief hypoxia, there may be a relative suppression of arousal responses. Early studies showed that healthy subjects frequently fail to arouse in NREM sleep during slowly progressive hypoxia even when SaO2 was reduced to 70%.6 Similarly, clinical observations in patients with neuromuscular or chest wall disease show these patients frequently spend prolonged periods of sleep with severe hypoxemia and no arousal. Furthermore, prolonged isocapnic hypoxia has been shown to release a number of inhibitory neurotransmitters and neuromodulators in the central nervous system, such as gamma aminobutyric acid (GABA), adenosine, and endogenous opioids.7,8 These inhibitory neurochemicals are likely responsible for the decline or “roll-off” in ventilatory drive during sustained and repetitive isocapnic hypoxia6,9 and may also be responsible for suppression of respiratory load sensation,10 respiratory-related evoked responses,11 and more general neuropsychologic functioning.12 To the extent that sleep arousal in general is dependent on intact central nervous system functioning and that respiratory-related arousals may importantly depend on the quantum of respiratory drive and associated sensations,12 prolonged hypoxia thus has the potential to suppress arousal either in general or, more specifically, to respiratory stimuli via depression of afferent or efferent respiratory pathways. Hypoxic depression of arousal in general or specifically to respiratory load would promote greater inspiratory effort at arousal and potentially greater postarousal ventilatory overshoot and subsequent undershoot in individuals exposed to sustained hypoxia, such as at altitude and in diseases such as chronic obstructive pulmonary disease, OSA, and sleep hypoventilation syndromes. Therefore, while sustained hypoxia has other sleep-disruptive effects (e.g., periodic breathing) and may coexist with important disease-specific phenomena that may also influence arousal (e.g., increased breathing effort), blunted arousal with hypoxia nevertheless has the potential to further destabilize respiratory control during sleep.

To test the hypothesis that hypoxia depresses neural mechanisms subserving arousal in general, we examined the susceptibility of normal subjects to arouse to auditory stimuli presented in
stage 2 and slow-wave sleep (SWS) on separate nights when subjects breathed either air or a gas mix to maintain mild isocapnic hypoxia (SaO₂, ~90%). Data obtained during these experiments on the cardiovascular responses to arousal were presented in an earlier paper.17

METHODS

Subjects

Following approval from the Repatriation General Hospital Research and Ethics Committee, 11 healthy nonsmoking men gave informed written consent and were enrolled in the study. Four subjects had previously participated in daytime studies involving brief hypoxia, but none had participated in overnight studies. The physical characteristics of the subjects were as follows: age, 20.3 ± 0.4 years; weight, 72.1 ± 2.4 kg; height, 179.0 ± 1.8 cm; body mass index, 22.5 ± 0.7 kg/m²; forced expiratory volume in the first second, 111.0% ± 6.7% of the predicted value; forced vital capacity, 101.4% ± 5.1% of the predicted value.

General Procedures

Each subject visited the laboratory on 3 separate nights, 1 week apart. Subjects slept in a temperature-controlled bedroom between 11:00 PM and 6:00 AM and were monitored from another room. Subjects breathed air (normoxia) on 1 night and a hypoxic gas mixture (hypoxia) on 2 nights with the treatment order randomized between subjects. Two hypoxic nights were employed in anticipation that hypoxia would disturb sleep and make data collection during stable sleep more difficult. Subjects were instructed to abstain from caffeine and alcohol consumption and strenuous exercise for 8 hours prior to each study.

Auditory Interventions

Transient arousals from stable NREM sleep were induced using brief auditory stimuli presented via ear-insert headphones (E-A-R-Tone, Cabot Safety Corporation/Auditory Systems Division, Indianapolis, IN). Electroencephalography (EEG) was monitored continuously (see below) to ascertain the onset and stability of sleep. Subjects were administered auditory tones and sham tones (no tone) repeatedly throughout the night following at least 30 seconds of stable stage 2 or SWS without arousal. Ten graded auditory tones (5-second duration; 4-dB increments; range, 54-90 dB; Cool Edit, Syntrillium Software Corporation, Phoenix, AZ) were given in an order that was randomized between subjects but essentially reproduced within subjects between nights. This was to ensure that comparable tones were administered at approximately equivalent times of night and that approximately equal numbers of tones within specific tone-intensity categories were presented between nights. Sham tones served to allow estimation of arousal probability in the absence of tone interventions and as controls for cardiovascular measurements.17 Auditory interventions were categorized into shams (no tone) or low (54-66 dB), medium (70-78 dB), or high (82-90 dB) intensities. The ratio of sham to auditory interventions was approximately 4:10.

Gas Delivery

Subjects breathed through a snug-fitting facemask and unidirectional breathing valve (8900 series, Hans-Rudolph, Kansas City, MO). The mask was sealed to the face with double-sided adhesive tape (Stomahesive Wafer C3709B, ConvaTec, Bristol-Myers Squibb Company, Princeton, NJ) and secured in place with a retaining cap (Hans Rudolph). Prior to each experiment, mask leaks were detected and corrected when necessary, by having the subject exhale against an occluded expiratory port. The mask inspiratory port was connected via tubing (Clean-Bor, Vacumed, length 1 m, ID 35 mm) to a T-piece in series with a 300 L foil bag (Scholle Industries, SA, Australia). The remaining arm of the T-piece incorporated a butterfly valve open to room air so that the position of the valve controlled the room air fraction (~0%-50%) of the inspirate.

During hypoxia nights, the foil bag was maintained close to full with 9% O₂ in N₂, and the butterfly valve adjusted to maintain SaO₂ within 80% to 90% during sleep. Hypoxia was induced gradually over a 10- to 15-minute period to minimize the potential for the hypoxic ventilatory response to disrupt sleep. No attempt was made to control end-tidal PCO₂. During rapid eye movement (REM) sleep, or any periods of unstable breathing, the inspired O₂ fraction was increased to prevent transient falls in SaO₂ below 80%. During normoxia nights, the foil bag was filled with air, and the butterfly valve was fully open to room air.

MEASUREMENTS

Subjects were instrumented, and continuous overnight polysomnography recordings made of EEG (C3/A2 and C4/A1), left and right electrooculogram, submental electromyogram, ECG (Hewlett-Packard 78342, Andover, MA), chest and abdominal movements (Vitalog Respiration, Redwood City, CA), arterial blood oxygen saturation (SaO₂) from the ear (POET II model 602-1, Criticare Systems, Inc., Waukesha, WI) and mask end-tidal CO₂ (PETCO₂; POET II model 602-3, Criticare Systems, Inc.) and sound intensity (Model NL-05 integrating sound level meter, Rion Co. Ltd., Japan) using a Compumedics (S-series, Abbotsford, Vic, Australia) data-acquisition system at a sampling rate of either 5 (SaO₂) or 125 (EEG, electrooculogram, electromyogram, electrocardiogram) Hz per channel.

Data Analysis

Sleep Staging and Arousal Scoring

A single trained observer (SC) blinded to the study gas and the sound-intensity channel performed the EEG analysis. Sleep stages were scored according to standard criteria.18 Arousal responses were assessed by reviewing the 30-second segment of the EEG record (C3/A2, C4/A1) following each tone or sham presentation. Arousals anywhere in the 30-second segment were scored according to previously published criteria19 and given a score according to the frequency and duration of EEG changes. A score of 0 was given if there was no discernible change in EEG frequency, 1 if there was an increase in delta activity, 2 if there was an increase in EEG frequency lasting 1.5 to 3 seconds, 3 if there was an increase in EEG frequency lasting 3 to 10 seconds, 4 if there was a 10- to 15-second increase in EEG frequency, and 5 if there was a greater than 15-second increase in EEG frequency. For each gas (normoxia and hypoxia) and sleep stage (stage 2 or stage 3/4) within each subject, a mean arousal score was determined for each tone category. In addition, the probability of a >3-second (arousal score 3-5) EEG arousal within each tone category
was calculated by dividing the number of presentations associated with arousal by the total number of tone presentations within the respective tone category for each gas and sleep stage within each subject. Tone intensities associated with a 50% probability of arousal from stage 2 sleep were derived from linear regression analyses of mean tone intensity versus arousal probability in each tone category during normoxia and hypoxia within each subject. A meaningful similar analysis was not possible in SWS, as only 3 subjects achieved greater than 50% arousal probability under both conditions in this stage.

The concordance between the trained observer (SC) and another experienced sleep technician who staged a subsample of the records was 88%. The intraobserver repeatability determined by restaging records was 94%. Equivalent interobserver and intraobserver repeatability were obtained in arousal classifications (87% and 90%, respectively).

**Statistical Analysis**

The effects of gas and sleep stage (stage 2 vs SWS) on arousal score and arousal probability to sham tones was examined using analysis of variance for repeated measures. The effects of gas, sleep stage, and tone intensity on arousal score and probability were examined using linear mixed model analysis (SPSS version 12, SPSS, Inc., Chicago, IL).20 Gas, sleep stage, and the 3 tone groups (54-66, 70-78, and 82-90 dB) were treated as fixed repeated factors, and the average measured tone intensity corresponding to each measurement of arousal probability was treated as a covariate to adjust for differences in average tone intensity between subjects, gas conditions, and sleep stages, using a scaled identity covariance matrix. Sleep parameters were compared between gases using Student paired t tests. All data are reported as means ± SEM. Null hypotheses were rejected when p < .05.

**RESULTS**

**SaO2 and PetCO2**

The mean SaO2 in the 10 minutes preceding all tone or sham presentations was 97.9% ± 0.2% on normoxia nights and 88.8% ± 0.4% on hypoxia nights (p < .001). Less than 1% of the night was spent with SaO2 below 95% during normoxia nights. In contrast, SaO2 was below 95% for approximately 60% of the night and below 90% for approximately 30% of the night during hypoxia nights.17 The mean PetCO2 determined from the 20 seconds preceding all tone and sham presentations was not different between normoxia and hypoxia nights (normoxia; 37.5 ± 1.3 mm Hg, hypoxia; 37.6 ± 0.8 mm Hg, p = .922).

**Tone Presentations, Sleep Architecture and Arousals**

The numbers of sham and tone presentations during stage 2 and SWS on normoxia and hypoxia nights are presented in Table 1. Sham and tone presentations were approximately evenly distributed between the sleep stages and tone groups in each gas condition, with the selected tone categories producing an approximately equal number of shams and tones per tone group (Table 1). The total number of sham and tone presentations was greater on the 2 combined hypoxia compared with the normoxia night (total hypoxia 21 ± 2 sham and 54 ± 6 tones versus total normoxia 15 ± 1 sham and 40 ± 3 tones, p < .05). However, in association with the requirements for stable periods of hypoxia, the average number of shams and tones on hypoxia nights (10 ± 1 sham, 27 ± 3 tones) was fewer compared with the normoxia night (sham p = .007, tones p = .002). There were no differences between normoxia and hypoxia nights in any of the sleep-architecture parameters investigated (Table 2).

The arousal score and probability of a longer than 3-second arousal as a function of tone intensity, gas condition, and sleep stage are presented in the Figure. Both the mean arousal score and the probability of a longer than 3-second arousal significantly increased with increasing tone intensity (p < .001) and were in the order of 40% to 60% lower during SWS compared with stage 2 sleep for any given tone intensity (stage effect p < .001), including sham tones (p = .011). Although there were strong trends towards significant gas × sleep-stage effects for both arousal score (p = .051) and the probability of arousal (p = .055), this reflected a combination of weak trends toward higher overall values in stage 2 sleep during hypoxia and lower values in SWS during hypoxia compared with normoxia, with no significant effect of gas within each sleep stage. There were no main effects and no other interaction effects involving gas condition. Similarly, there was no difference between normoxia and hypoxia in the calculated tone intensity associated with a 50% probability of arousal from stage 2 sleep (83 ± 15 dB versus 80 ± 14 dB, p = .697).

**DISCUSSION**

The main finding of this study was that mild overnight hypoxia did not appear to alter auditory arousability from sleep in young healthy subjects. Although for safety and ethical reasons the hypoxia and auditory arousal from NREM sleep—Catcheside et al
Hypoxia was maintained throughout the night, such that the cumulative degree of hypoxia was not dissimilar to that exhibited by many patients with mild to moderate respiratory disturbance in sleep, including patients with OSA, who are known to exhibit impaired arousal responses to airway occlusion. Furthermore, overnight mild hypoxia (SaO2 ~90%) has previously been shown to produce significant hypoxic ventilatory roll-off during NREM sleep in healthy subjects, indicating that mediators of hypoxic ventilatory depression and potentially other central nervous system depressant effects are released with this degree of hypoxia.

We also believe it is unlikely that a clinically meaningful effect of mild hypoxia on auditory arousability from sleep was missed as a result of type II error. Based on average within-subject standard deviations in arousal score and arousal probability of 0.62 and 0.18 units respectively, obtained from measurements within each tone category and sleep stage on the 2 separate hypoxia nights, we estimate that hypoxia-related changes in the order of 0.8 (arousal score units) and 0.2 (probability units), respectively, would have been detected with a power of 0.8 and 2-tailed significance level of .05. Consequently, in the absence of any gas condition-related trends, we believe the likelihood of having missed an important systematic effect of mild hypoxia on auditory arousability is relatively low. However, it remains clearly possible that with more severe hypoxia, such as may occur in chronic obstructive pulmonary disease, respiratory failure and severe OSA, arousal to auditory and other stimuli may nevertheless show hypoxic impairment.

The neural circuits underpinning arousal are complex, involving several brainstem regions, including the locus coeruleus and raphe, pedunculopontine, and laterodorsal tegmental nuclei, and several primary neurotransmitters, including norepinephrine, serotonin, and acetylcholine. Given that hypoxia did not appear to influence the likelihood or magnitude of arousal to auditory stimuli in the present study, it would appear that these systems are relatively insensitive to the central nervous system depressive effects of at least mild hypoxia.

Sensory gating during sleep appears to be stimulus and sleep-stage dependent. A wide range of stimuli, such as auditory, vibrotactile, added inspiratory resistance, olfactory, and brief nociceptive stimuli demonstrate more-frequent and intense arousals with increasing stimulus intensity and, in general, reduced responsiveness in SWS compared with in lighter sleep. On the other hand, longer-duration nociceptive stimulation via intramuscular hypertonic infusion has been shown to elicit waking responses with similar frequencies in stage 2, SWS, and REM sleep, consistent with differences in prearousal sensory processing between stimulus modalities and/or time-dependent integration of sensory inputs to the arousal system. Data from Afifi and Colrain, showing sleep-specific dampening of respiratory-related but not auditory-evoked potentials in OSA patients compared with control subjects, supports that there are important differences in the central processing of auditory versus respiratory-load information that are not apparent in wakefulness. Combined with recent data consistently showing that hypoxia dampens respiratory-load sensations awake, this suggests that arousal to inspiratory load during sleep, which may depend on similar sensory pathways, may show a similar respiratory-specific vulnerability to hypoxic depression. Although Gleeson et al. found little evidence for a higher respiratory effort at arousal with a hypoxic compared with a hypercapnic or resistive load stimulus, only three 2-minute acute hypoxic episodes were administered overnight. Based on the time course of hypoxic ventilatory depression, this is not sufficient for elaboration of the cumulative depressant effects of more-sustained hypoxia.

Our group has consistently shown hypoxia to blunt respiratory-load perception during wakefulness, consistent with hypoxic depression of neural processes involved in the transduction and/or central processing of respiratory sensations. Results from a recent neurophysiologic study to further investigate these findings revealed that the P1 and P2 components of the respiratory-related evoked potential to well-matched midinspiratory resistive loads were bluntly during sustained mild to moderate hypoxia (SaO2~80%) compared with normoxia. Should respiratory mechanoreceptor-dependent inputs to the arousal system relay via shared or similar hypoxia-sensitive circuits involved in respiratory mechanoreception, then greater respiratory effort may be required in order to initiate arousal. Thus, while data from the current study suggest that mild hypoxia does not impair audition and the arousal system itself during sleep, the influence of sus-
tained hypoxia on respiratory mechanoreceptor-mediated arousal responses remains to be elucidated.

Methodologic Considerations

In this study, we elected to deliver a range of tones in random order during stable sleep rather than employing replicate trials of escalating tone intensity within a short duration, as has been used in several previous studies. Although an escalating-tone protocol has the advantage of allowing multiple measurements of the tone intensity that reliably elicits arousal (i.e., auditory arousal threshold), it does not allow for response comparisons between a broad range of tone intensities that are free from potential carry-over effects from previous tones. The protocol used in this study was therefore chosen specifically to help establish the potential influence of hypoxia on a range of arousal responses from established stable sleep.

Although tone delivery was continued in REM sleep, few subjects exhibited sufficient consolidated REM sleep for multiple tone presentations, with only 5 subjects receiving more than 1 to 2 presentations in each tone category. Consequently, meaningful comparisons between gas conditions and with NREM sleep were not possible, and the effects of hypoxia on acoustic arousability from REM sleep remain unknown.

In summary, these findings suggest that mild hypoxia does not impair the neural mechanisms involved in arousal from sleep to auditory stimuli. The potential for sustained hypoxia to depress respiratory mechanoreceptor-mediated arousal remains to be investigated.

REFERENCES