Impact of repeated daily exposure to intermittent hypoxia and mild sustained hypercapnia on apnea severity

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Yokhana SS, Gerst DG 3rd, Lee DS, Badr MS, Qureshi T, Mateika JH. Impact of repeated daily exposure to intermittent hypoxia and mild sustained hypercapnia on apnea severity. J Appl Physiol 112: 367–377, 2012. First published November 3, 2011; doi:10.1152/japplphysiol.00702.2011.—We examined whether exposure to intermittent hypoxia (IH) during wakefulness impacted on the apnea/hypopnea index (AHI) during sleep in individuals with sleep apnea. Participants were exposed to twelve 4-min episodes of hypoxia in the presence of sustained mild hypercapnia each day for 10 days. A control group was exposed to sustained mild hypercapnia for a similar duration. The intermittent hypoxia protocol was completed in the evening on day 1 and 10 and was followed by a sleep study. During all sleep studies, the change in esophageal pressure (∆Pes) from the beginning to the end of an apnea and the tidal volume immediately following apneic events were used to measure respiratory drive. Following exposure to IH on day 1 and 10, the AHI increased above baseline measures (day 1: 1.95 ± 0.42 fraction of baseline, P = 0.01, vs. day 10: 1.53 ± 0.24 fraction of baseline, P < 0.06). The indexes were correlated to the hypoxic ventilatory response (HVR) measured during the IH protocol but were not correlated to the magnitude of ventilatory long-term facilitation (vLTF). Likewise, ∆Pes and tidal volume measures were greater on day 1 and 10 compared with baseline (∆Pes: −8.37 ± 0.84 vs. −5.90 ± 1.30 cmH2O, P = 0.04; tidal volume: 1.193.36 ± 101.85 vs. 1,01.14 ± 0.83 ml, P = 0.01). This was not the case in the control group. Interestingly, the AHI on day 10 (0.78 ± 0.13 fraction of baseline, P = 0.01) was significantly less than measures obtained during baseline and day 1 in the mild hypercapnia control group. We conclude that enhancement of the HVR initiated by exposure to IH may lead to increases in the AHI during sleep and that initiation of vLTF did not appear to impact on breathing stability. Lastly, our results suggest that repeated daily exposure to mild sustained hypercapnia may lead to a decrease in breathing events.

progressive augmentation; ventilatory long-term facilitation; apnea/hypopnea index; apnea duration; esophageal pressure

EXPOSURE TO INTERMITTENT HYPOXIA leads to the initiation of various forms of respiratory motor plasticity, including progressive augmentation of the hypoxic ventilatory response (HVR) and long-term facilitation (26, 30, 32, 35, 43). Progressive augmentation is characterized by a gradual increase in the HVR in the course of advancement from the initial to the final hypoxic episode of an intermittent hypoxia protocol (30, 43). Enhancement of the HVR can be sustained long after exposure to acute intermittent hypoxia (14, 29). Long-term facilitation is a form of neuronal plasticity that is characterized by a progressive increase in respiratory motor output during periods of normoxia that are separated by hypoxic episodes and by a sustained elevation in respiratory motor activity for up to 90 min following exposure (26, 30, 32, 34). In many animals (32), a first-time exposure to intermittent hypoxia initiates long-term facilitation of the hypoglossal nerve and the genioglossus muscle (14, 28, 33), phrenic motoneurons (1, 18), and minute ventilation or its components (i.e., tidal volume and/or breathing frequency; Refs. 40, 50, 52–54). Repeated daily exposure to intermittent hypoxia enhances the magnitude of long-term facilitation at these sites (25, 42, 45) and initiates long-term facilitation of carotid sensory nerve activity (41). Recent studies have shown that an acute exposure to intermittent hypoxia initiates progressive augmentation and long-term facilitation of minute ventilation (vLTF) and genioglossus muscle activity in healthy humans (6, 17, 24) and in humans with sleep apnea (15, 24). Likewise, the magnitude of vLTF can be enhanced by repeated daily exposure to intermittent hypoxia in humans with sleep apnea (15).

Given that individuals with sleep apnea are exposed to intermittent hypoxia throughout the night, it is possible that progressive augmentation and long-term facilitation are initiated naturally during sleep. If this hypothesis is correct, the question remains as to whether or not the initiation of these forms of respiratory plasticity are beneficial or a detriment to breathing stability (30). On the one hand, augmentation of the ventilatory response to hypoxia might promote increases in apneic events by reducing carbon dioxide levels below the apneic threshold (30). The induction of hypocapnia is most obvious following an apneic event; whereby an inappropriately high ventilatory response to hypoxia and hypercapnia generated during a preceding apnea induces hypocapnia that results in the initiation of a subsequent apnea (9, 10). Moreover, this sequence of events likely results in the generation of both central and obstructive events. There is strong evidence that reducing carbon dioxide below the apneic threshold initiates a centrally mediated apnea followed by obstruction of the upper airway, presumably as a consequence of the disfacilitation of upper airway muscles via hypocapnia (2, 3). On the other hand, vLTF and long-term facilitation of upper airway muscle activity might promote breathing stability and mitigate apneic events, particularly if these forms of plasticity are sustained in the presence of hypocapnia (30). If vLTF can be sustained in the presence of the hypocapnia, this would prevent the development of central apneas (30), while the induction of long-term facilitation of upper airway muscles in individuals with sleep apnea would prevent the development of obstructive events (30). However, the impact of progressive augmentation and vLTF on apnea severity has not been previously investigated. To fill this void, we measured breathing events during sleep in...
individuals with sleep apnea, immediately following exposure to intermittent hypoxia during wakefulness. These measures were obtained after an initial exposure and following repeated daily exposure to intermittent hypoxia. This study was one component of an investigation that was also designed to determine the impact of time of day and repeated daily exposure to intermittent hypoxia on the magnitude of progressive augmentation and vLTF during wakefulness. The results from this concurrent investigation have been published previously (15).

METHODS

Protocol

The Human Investigation Committees of Wayne State University School of Medicine and John D. Dingell Veterans Affairs Medical Center approved the experimental protocol. Twenty-six participants with both pure obstructive and predominantly obstructive (i.e., a central component combined with an obstructive component) sleep apnea but no other comorbidities (e.g., heart and lung disease, hypertension, and obesity) completed the protocol. In addition, four other participants were enrolled in the study but because of unforeseen time constraints did not complete the protocol. Consequently, the data from these participants were not included in the analysis.

The 26 participants that completed the protocol visited the laboratory on 12 occasions. During the first visit to the laboratory, written informed consent was obtained and thereafter a physical examination, health and lifestyle questionnaires, blood pressure measurements, and a 12-lead EKG were completed. After ensuring that all inclusion criteria were met, participants were exposed to two 4-min episodes of hypoxia to ensure familiarization with the experimental protocol and apparatus (Fig. 1). During the second visit to the laboratory, participants completed a baseline nocturnal polysomnogram to confirm the presence of obstructive sleep apnea (Fig. 1). Upon verification of the presence of sleep apnea, participants were enrolled into the protocol.

Thirteen of the twenty-six participants were exposed to intermittent hypoxia (see Intermittent hypoxia protocol for description) during visits 3–12 to the laboratory. From here on, the participants exposed to intermittent hypoxia will be referred to as the experimental group and visits 3 and 12 will be referred to as day 1 and 10 of the protocol (Fig. 1). The remaining 13 participants were exposed to a sham protocol that was identical to the actual protocol with the exception that participants were not exposed to intermittent hypoxia. From here on these participants will be referred to as the control group. Exposure to the intermittent hypoxia and sham protocol occurred while participants were awake. The participants in the control group were matched to the participants in the experimental group on the basis of age, race, and body mass index. On day 1 and 10, participants came to the laboratory between 6 and 7 PM and were exposed to the intermittent hypoxia or sham protocol. Thirty minutes following completion of either protocol the participants completed a sleep study (Fig. 1). These studies, in addition to the baseline sleep study, were initiated at the same time of night to eliminate the potential impact that circadian rhythms might have on nocturnal breathing events. On days 2–9, participants were exposed to the intermittent hypoxia or sham protocol in the morning (7–8 AM) (Fig. 1). Participants were instructed to avoid food and caffeinated beverages 4–6 h before each study. All studies were completed in a quiet environment to eliminate the impact of environmental noise (e.g., talking, phone calls) on measures of ventilation.

During the intermittent hypoxia and sham protocol, participants assumed a supine position throughout the study and the required monitoring equipment was attached (see Instrumentation for details). At the beginning of both the intermittent hypoxia and sham protocols, participants breathed room air for 10 min to establish baseline values of minute ventilation, end-tidal partial pressure of oxygen ($P_{ET}O_2$), and end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) (Fig. 1). From this point forward this initial baseline period will be referred to as baseline 1 ($B_1$). Thereafter, $P_{ET}CO_2$ was raised 3 Torr above $B_1$ values and the variables outlined above were recorded for an additional 15

![Intermittent Hypoxia Protocol](image-url)
From this point forward this period will be referred to as baseline 2 (B2). The PETCO2 was sustained at B2 levels throughout the remainder of both the intermittent hypoxia and sham protocols. Following B2, participants that completed the intermittent hypoxia protocol were exposed to twelve 4-min episodes of hypoxia, wherein PETCO2 was maintained at 50 Torr (Fig. 1). Each hypoxic episode was terminated with a single breath of 100% O2 to rapidly bring the PETO2 to the normoxic range. Each episode, with the exception of the 12th episode, was followed by a 4-min recovery period during which time participants breathed room air. The final recovery period following the 12th hypoxic episode lasted for 30 min (Fig. 1). Participants that completed the sham protocol inspired compressed air in place of the hypoxic gas mixture during twelve 4-min episodes. At the end of each sham episode, participants were exposed to a single breath of 100% oxygen followed by 4 min of breathing room air with the exception of the last episode, which was followed by a 30-min recovery period.

Instrumentation

Nocturnal polysomnography. During completion of the sleep studies (i.e., baseline, day 1, and day 10), the sleep-monitoring montage included an electroencephalogram (C3/A2, C4/A1, O1/A2, and O2/A1), electrooculogram, submental electromyogram, and an electrocardiogram (EKG). Abdominal and chest wall movements were monitored using a piezoelectric band, and esophageal pressure (Pes) was measured using a pressure transducer (model MPC-500; Millar Instruments, Houston, TX). Airflow, breathing frequency, and inspiratory/expiratory volume were recorded via a pneumotachograph (model RSS-100HR; Hans-Rudolph, Kansas City, MO) that was connected to the flowmeter. Thus supplemental oxygen and carbon dioxide could be added to the 8% oxygen/balance nitrogen that was inspired during a given 4-min hypoxic episode to maintain carbon dioxide levels near baseline 1 (B1). PETCO2 was sustained at B1 levels throughout the remainder of both the intermittent hypoxia and sham protocols. Following B1, participants that completed the intermittent hypoxia protocol were exposed to twelve 4-min episodes of hypoxia, wherein PETCO2 was maintained at 3 Torr above baseline (PETCO2 = 3 Torr above baseline) (Fig. 1). Each hypoxic episode was terminated with a single breath of 100% O2 to rapidly bring the PETO2 to the normoxic range. Each episode, with the exception of the 12th episode, was followed by a 4-min recovery period during which time participants breathed room air. The final recovery period following the 12th hypoxic episode lasted for 30 min (Fig. 1). Participants that completed the sham protocol inspired compressed air in place of the hypoxic gas mixture during twelve 4-min episodes. At the end of each sham episode, participants were exposed to a single breath of 100% oxygen followed by 4 min of breathing room air with the exception of the last episode, which was followed by a 30-min recovery period.

Data Analysis

Nocturnal polysomnography. Data collected during the initial 3 h of the sleep study were compared between studies (baseline vs. day 1 vs. day 10). These hours were selected for comparison because we reasoned that if progressive augmentation and/or VLTF initiated during wakefulness impacted on breathing stability it would be most obvious in the hours immediately following exposure. Likewise, we surmised based on previous experience that the duration and quality of sleep would be best early on in the sleep period given the instrumentation employed.

Sleep stages and arousals were scored according to standard criteria (20). An apnea was defined as the absence of inspiratory airflow for a minimum of 10 s. A hypopnea was defined as an event accompanied by a >30% reduction in airflow that lasted 10 s accompanied by a ≥5% reduction in SaO2. We chose this latter criterion to ensure that all obvious breathing events were detected. Central and obstructive events were differentiated according to standard criteria (20). A mixed apnea was characterized by the absence of airflow and esophageal pressure excursions (i.e., respiratory effort) at the commencement of the event, followed by a resumption of respiratory effort that was associated with esophageal pressure measures that progressively became more negative. A mixed apnea or hypopnea was also designated if the absence or reduction of airflow at the onset of an event was accompanied by a gradual decrease in respiratory effort that was associated with an esophageal pressure pattern that gradually became less negative before a progressively increasing respiratory effort, without a concomitant rise in ventilation, was evident.

The total number of arousals, as well as obstructive, mixed, and central breathing events detected during nonrapid eye movement sleep, was calculated for the selected time frame (i.e., initial 3 h). Thereafter, these measures, in addition to the total apnea/hypopnea index, were expressed per hour of sleep. In addition, the duration of apneas and the ratio of apneas or hypopneas to the total number of breathing events were determined. Further measures of sleep disorder severity were obtained by calculating the lowest value of SaO2 during the sleep period, the mean SaO2 measured from the detected respiratory events, and the number of minutes above an SaO2 value of 90%.

Secondary measures of respiratory drive and arousal were also determined. More specifically, baseline measures of tidal volume, SaO2, PETCO2, Pes, and inspiratory and expiratory flow were measured from five breaths recorded during stable breathing (i.e., free of apneas and hypopneas) at the onset of the 3-h segment of sleep. During airway occlusion (i.e., apnea) swings in Pes were measured and the following indexes were determined: the least negative inspiratory esophageal pressure at the start of an apnea (Pesmin), the most negative inspiratory esophageal pressure at the end of an apnea (Pesmax), and the difference between Pesmax and Pesmin. The lowest SaO2 during an apnea and the difference between baseline SaO2 and the lowest SaO2 immediately before and the minimum SaO2 during an apnea (baseline SaO2 − nadir SaO2 = ΔSaO2) were calculated. Likewise, tidal volume and PETCO2 were measured from the first three breaths recorded after the termination of an apnea. The longest and shortest EKG interbeat interval (i.e., R-R interval) during a breathing event was determined, as was the average interbeat interval for the inspiratory phase of the first three breaths recorded following apnea termination.

ΔPes, ΔPes/SaO2, ΔPes/Δt, and the tidal volume immediately following apnea termination were used as secondary indexes of respiratory drives (20). ΔSaO2/Δt were exposed to twelve 4-min episodes of hypoxia, wherein PETCO2 was maintained at 3 Torr above baseline (PETCO2 = 3 Torr above baseline) (Fig. 1). Each hypoxic episode was terminated with a single breath of 100% O2 to rapidly bring the PETO2 to the normoxic range. Each episode, with the exception of the 12th episode, was followed by a 4-min recovery period during which time participants breathed room air. The final recovery period following the 12th hypoxic episode lasted for 30 min (Fig. 1). Participants that completed the sham protocol inspired compressed air in place of the hypoxic gas mixture during twelve 4-min episodes. At the end of each sham episode, participants were exposed to a single breath of 100% oxygen followed by 4 min of breathing room air with the exception of the last episode, which was followed by a 30-min recovery period.

Impact of intermittent hypoxia on apnea severity

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for each group because measures of Pes from each sleep study (i.e., baseline, day 1 and day 10) were not available due to technical difficulties or because the number of apneas within the initial 3 h of sleep from one or more of the studies was insufficient to include in the analysis.

**Intermittent hypoxia protocol.** For day 1 and 10 the following measures were obtained. Average values of minute ventilation, PETCO₂, PETO₂, and Sao₂, henceforth referred to as the physiological variables, were determined for the last 5 min of B₁. Average values for the measured physiological variables were also obtained for the last 5 min of B₂ and for the last 2 min of the 12 hypoxic episodes that followed. To quantify the HVR on day 1 and 10, the change in minute ventilation from the baseline period immediately before the 11th hypoxic episode to the last 2 min of the 11th hypoxic episode (H₁₁) was divided by the change in Sao₂ from the baseline period to the last 2 min of the episode. This calculation was also completed for the 12th hypoxic episode. The results obtained from the 11th and 12th episodes were averaged.

In addition to the average values calculated for the baseline periods and hypoxic episodes, average values were also calculated for the last 2 min of each recovery period except the end-recovery period. For presentation purposes, the 30 min end-recovery period was divided into six 5-min segments and the physiological variables were averaged for each of these segments. For the purposes of statistical analysis, a single average for the 30-min recovery period was obtained, since no obvious differences in each of the measured variables were evident between segments. For day 1 and 10 of the protocol, minute ventilation recorded during the recovery periods was expressed in both absolute values and as a fraction of baseline. We also calculated a single average value using the data recorded during B₂ and each recovery period on day 1 and 10. This latter analysis was completed to compare the results between the experimental and control groups.

**Sham protocol.** The sham protocol was completed primarily to compare the magnitude of minute ventilation during the recovery periods to measures obtained during the intermittent hypoxia protocol. This comparison was made to ensure that vLTF was a consequence of exposure to intermittent hypoxia and not other variables (i.e., sustained carbon dioxide). The data collected during the sham protocol was analyzed at identical time points to the outlined from the intermittent hypoxia protocol. The values determined for B₂ and each recovery period on day 1 and day 10 were averaged since the response to the sham protocol on each day was identical.

**Statistical analysis.** An unpaired t-test was used to determine whether age, height, weight, body mass index, baseline apnea/hypopnea index, apnea duration, blood pressure, and scores derived from the Epworth and Stanford sleepiness scales were different between the groups. A one-way ANOVA was completed to determine if the apnea/hypopnea index and apnea duration differed among baseline, day 1, and day 10 within the experimental and control groups. This analysis was completed since the results from the unpaired t-test indicated that the baseline apnea/hypopnea indexes and duration tended to be different between groups. Thereafter, the apnea/hypopnea index and apnea duration determined on day 1 and 10 were standardized relative to baseline measures. A two-way ANOVA with repeated measures was used to compare the standardized apnea/hypopnea index or apnea duration on day 1 and 10 within and between the experimental and control groups. A two-way ANOVA with repeated measures was also used to determine if 1) the total sleep time and time in a given sleep stage, 2) the apnea or hypopnea ratio (the number of apnea or hypopneas divided by the total number of breathing events), and 3) the central, obstructive, or mixed apnea index, differed among baseline, day 1, and day 10 within and between the experimental and control groups. This analysis was also employed to compare 4) minute ventilation, PETCO₂, and PETO₂ during B₁ on day 1 and 10 of the intermittent hypoxia or sham protocol, and 5) tidal volume and PETCO₂ measures obtained during stable sleep and following apneic events during the sleep studies completed at baseline and on day 1 and 10 in the experimental or control group. A three-way ANOVA with repeated measures was used to compare minute ventilation, PETCO₂, and PETO₂ during B₂ and end recovery on day 1 and 10 of the intermittent hypoxia or sham protocol. Fisher least significant difference post hoc test was used in conjunction with all analysis of variance tests completed.

The changes in respiratory drive (e.g., ΔPes/ΔSao₂) and arousal (e.g., ΔEKG during apnea) indexes during sleep on day 1 and day 10 relative to baseline were similar. Consequently, the data from day 1 and 10 were averaged and a paired t-test or a signed rank test, if the data were not normally distributed, were used to determine if differences between the average data from day 1 and 10 differed from baseline within the experimental (n = 9) and control (n = 8) groups. Between group comparison were not completed because an insufficient number of matched pairs with complete data from all trials was available (n = 5; see Data Analysis: Nocturnal polysomnography for explanation) and because the data in some cases were not normally distributed.

A Pearson product moment correlation was used to correlate the average HVR (determined from the last 2 episodes of the intermittent hypoxia protocol) and the magnitude of vLTF (determined from the end-recovery period) to the apnea/hypopnea index measured during the initial 3 h of sleep on day 1 and 10. All results are presented as means ± SE. A value of P ≤ 0.05 was considered significant, while 0.05 < P < 0.08 was considered to reflect a trend toward significance.

**RESULTS**

The minute ventilation data and corresponding Sao₂, PETO₂, and PETCO₂ values collected during wakefulness from the experimental and control group on day 1 and 10 are similar to results published previously (15). The only difference is that data from five participants have been added to the control group. Given that findings from the experimental group are identical to our previous publication (15) and that the differences between the experimental and control group are qualitatively similar (but not quantitatively identical), we have not duplicated the results by request of the American Physiological Society Publication Committee. However, we have guided the reader (see below) to those figures in our previous publication that illustrate that progressive augmentation and vLTF was initiated in response to intermittent hypoxia, and that the magnitude of vLTF was greater following intermittent hypoxia compared with the sham protocol. The results obtained from the sleep studies (i.e., day 1 and 10) that are outlined below (see Respiratory Plasticity and Nocturnal Breathing Events) are novel and have not been published previously.

**Anthropometric Variables and Baseline Sleep Study Measures**

The age, body mass index, and race of the participants were similar between the groups. In general, the participants were young and had a body mass index indicating the absence of morbid obesity (Table 1). Systolic and diastolic blood pressures were within normal limits and were similar between the groups (Table 1). Completion of the Epworth sleepiness scale indicated a history of mild sleepiness in both groups, although scores from the Stanford sleepiness scale indicated otherwise on the day of the screening visit (Table 1). The measures of sleepiness were not significantly different between groups. The average Sao₂ during breathing events and the percentage of total sleep time that Sao₂ remained between 90 and 99% were
Table 1. Anthropometric and sleep measures from baseline sleep study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental Group</th>
<th>Control Group</th>
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<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27.1 ± 1.7</td>
<td>27.5 ± 1.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181.9 ± 2.2</td>
<td>178.0 ± 2.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.5 ± 3.2</td>
<td>83.6 ± 3.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2 ± 0.7</td>
<td>26.3 ± 0.7</td>
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<tr>
<td>Systolic pressure, Torr</td>
<td>119.0 ± 2.4</td>
<td>123.0 ± 1.2</td>
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<tr>
<td>Diastolic pressure, Torr</td>
<td>72.6 ± 1.7</td>
<td>75.3 ± 1.5</td>
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<tr>
<td>Epworth sleepiness scale</td>
<td>11.2 ± 0.9</td>
<td>9.0 ± 1.5</td>
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<tr>
<td>Stanford sleepiness scale</td>
<td>2.8 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Apnea/hypopnea index, events/h</td>
<td>32.1 ± 4.1*</td>
<td>43.0 ± 4.3</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>14.7 ± 0.8*</td>
<td>16.9 ± 1.0</td>
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<tr>
<td>Average SaO₂ during apnea, %</td>
<td>92.7 ± 0.5</td>
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<td>SaO₂ between 90–99%, %total sleep time</td>
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<td>99.4 ± 0.3</td>
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<tr>
<td>Race</td>
<td>AA 8</td>
<td>AA 8</td>
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<tr>
<td>Age, yr</td>
<td>27.1 ± 1.7</td>
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</tr>
<tr>
<td>Race</td>
<td>AA 8</td>
<td>AA 8</td>
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Values are means ± SE. SaO₂, oxygen saturation; AA, African American; C, Caucasian; H, Hispanic. Note that the apnea/hypopnea indexes and duration were obtained from the initial 3 h of sleep and not from the full night as reported in Ref. 15. *P ≤ 0.08, trend toward a difference between the experimental and control group.

similar in the experimental and control groups (Table 1). In contrast, despite matching for a variety of anthropometric variables, the apnea/hypopnea index and apnea duration at baseline tended to be less in the experimental group compared with the control group (apnea/hypopnea index, P ≤ 0.08; apnea duration P ≤ 0.07; Table 1). As shown in Table 2 the baseline apnea/hypopnea index was comprised primarily of obstructive and mixed events in the experimental and control groups.

Intermittent Hypoxia and Sham Protocol: B₁ and B₂, Hypoxia, and End Recovery

Minute ventilation, PěTO₂, and PěTCO₂, measured during B₁ of the intermittent hypoxia protocol on day 1 and 10 have been published previously (15) and are shown in Fig. 1 of the previous publication (see “Intermittent Hypoxia-Initial and Final PM”). Likewise, minute ventilation, PěTO₂, and PěTCO₂, measured during B₂ on day 1 and 10 are shown in Figs. 3 and 4 of our previous publication (see “B₂-Initial and Final Day-PM”; Ref. 15). The changes in minute ventilation that occurred during and following the intermittent hypoxia protocol on day 1 and 10 can be found in Fig. 7 of our previous publication (see “PM-Initial Day and PM-Final Day; Ref. 15). Note that a progressive increase in minute ventilation during the hypoxic episodes was evident throughout the protocol on day 1 and that the minute ventilation response to the initial hypoxic episode was significantly greater on day 10 compared with day 1, as was the HVR (see Fig. 5 in Ref. 15; “Initial and Final PM” for details), which is indicative of progressive augmentation. However, because minute ventilation did not progressively increase throughout the intermittent hypoxia protocol on day 10 the minute ventilation response during the final hypoxic episodes was similar on day 1 and 10. Also note that minute ventilation measured during the end-recovery period of the intermittent hypoxia protocol on day 10 (see Fig. 7; PM Initial Day and PM-Final Day” in Ref. 15 for specific details) was greater than B₂ values, which is indicative of vLTF. The PěTO₂, and PěTCO₂, values measured during end-recovery are shown in Fig. 4 of our previous publication (15).

Minute ventilation measured during the end-recovery period of the intermittent hypoxia and sham protocol on day 1 and 10 were significantly greater than B₂ values (e.g., see Fig. 8 in Ref. 15). However, minute ventilation measured during the end-recovery period of the intermittent hypoxia protocol was significantly greater than the values measured during the end-recovery period of the sham protocol (22.7 ± 0.9 vs. 17.9 ± 0.9 l/min, P = 0.003). The alterations in PěTO₂, and PěTCO₂, that were observed during the end-recovery periods of the intermittent hypoxia and sham protocols are outlined in the text of our previous publication (see “Intermittent hypoxia protocol vs. Sham protocol in Ref. 15”.

Table 2. Sleep architecture and breathing events from baseline, day 1, and day 10 sleep studies

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Control Group</th>
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<tbody>
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<td>Sleep period time, min</td>
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<td>179.4 ± 1.1</td>
</tr>
<tr>
<td>Total sleep time, min</td>
<td>1367.6 ± 9.1</td>
<td>1532.4 ± 4.0</td>
</tr>
<tr>
<td>Sleep efficiency, %total sleep time</td>
<td>80.8 ± 3.4</td>
<td>85.4 ± 2.2</td>
</tr>
<tr>
<td>%Total sleep time in stage N1</td>
<td>54.0 ± 8.0</td>
<td>48.6 ± 7.6</td>
</tr>
<tr>
<td>%Total sleep time in stage N2</td>
<td>41.8 ± 7.6</td>
<td>45.1 ± 6.3</td>
</tr>
<tr>
<td>%Total sleep time in stage REM</td>
<td>1.9 ± 1.3</td>
<td>4.7 ± 2.0</td>
</tr>
<tr>
<td>Apnea/hypopnea index, events/h</td>
<td>32.1 ± 4.1</td>
<td>46.6 ± 5.1†</td>
</tr>
<tr>
<td>Obstructive apnea/hypopnea index, events/h</td>
<td>14.4 ± 3.4</td>
<td>26.7 ± 5.2</td>
</tr>
<tr>
<td>Mixed apnea/hypopnea index, events/h</td>
<td>14.9 ± 2.8</td>
<td>18.0 ± 3.1</td>
</tr>
<tr>
<td>Central apnea/hypopnea index, events/h</td>
<td>2.7 ± 1.9</td>
<td>1.9 ± 1.6</td>
</tr>
<tr>
<td>Apnea ratio</td>
<td>0.19 ± 0.05</td>
<td>0.33 ± 0.07†‡</td>
</tr>
<tr>
<td>Hypopnea ratio</td>
<td>0.81 ± 0.05</td>
<td>0.67 ± 0.07†‡</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>14.7 ± 0.8</td>
<td>18.3 ± 1.3‡</td>
</tr>
<tr>
<td>Respiratory arousal index, events/h</td>
<td>21.5 ± 3.0</td>
<td>25.2 ± 3.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. N1, stage 1 of nonrapid eye movement sleep; N2, stage 2 of nonrapid eye movement sleep; N3, stage 3 (formally slow wave sleep) of nonrapid eye movement sleep; REM, rapid eye movement sleep. *Significantly different from the baseline and day 1 sleep study. †Trend toward a difference from baseline in the experimental group. ‡Significantly different from the control group. $Significantly different from the baseline sleep study. See text for P values.
Respiratory Plasticity and Nocturnal Breathing Events

Total sleep time, sleep efficiency, and time spent in a given stage of nonrapid eye movement sleep were not significantly different between baseline and day 1 and 10 when these measures were compared within or between the intermittent hypoxia and control group (Table 2).

The absolute apnea/hypopnea indexes measured from baseline, day 1 and 10 following the intermittent hypoxia and sham protocols are shown in Table 2. Within the experimental group the apnea/hypopnea index on day 1 (P = 0.01) and day 10 (P = 0.058) was greater than baseline measures, although this difference did not reach statistical significance on day 10 (Table 2). The increase in the apnea/hypopnea index was primarily due to increases in both obstructive and mixed events (Table 2). Within the control group, the apnea/hypopnea index on day 10 was significantly less than measures obtained at baseline and on day 1 (P = 0.01) (Table 2). Standardization of the data relative to baseline (see Statistical analysis for explanation) revealed that the increase in the apnea/hypopnea index observed on day 1 and 10 in the experimental group was significantly greater than the standardized apnea/hypopnea index measured from the control group (P = 0.02; Fig. 2). Likewise, the standardized data revealed that the apnea/hypopnea index on day 10 was less than the index on day 1 (P = 0.01) (Fig. 2). This finding was applicable to both groups since the results of the two-way ANOVA did not reveal an interaction effect. Nevertheless, this finding was more clearly evident in the control group since a reduction in the apnea/hypopnea index was evident in 11 of the 13 participants. This was not the case in the experimental group since a reduction in the apnea/hypopnea index was evident in only 8 of the 13 participants.

In addition to the increase in the apnea/hypopnea ratio, a small but significant increase in the duration of breathing events increased on day 1 (P = 0.003) and 10 (P < 0.001) in the experimental group (Table 2). Standardization of the data relative to baseline (see Statistical analysis for explanation) revealed that the increase in apnea duration on day 1 and 10 in the experimental group was significantly different from the control group (P = 0.02; Fig. 2). The increase in the apnea/hypopnea index observed on day 1 and 10 in the experimental group was also accompanied by changes in the apnea and hypopnea ratio (i.e., number of apneas or hypopneas divided by the total number of breathing events; see Table 2, apnea ratio and hypopnea ratio). The apnea ratio on day 1 (P = 0.08) and day 10 (P = 0.003) increased while the hypopnea ratio decreased (day 1: P = 0.08; day 10: P = 0.003) relative to baseline measures in the experimental group, although the change on day 1 did not reach statistical significance. These alterations in the apnea and hypopnea ratio were not evident in the control group. Consequently, the apnea and hypopnea ratios in the experimental group were significantly different from the control group on day 10 (P = 0.01). An increase in obstructive (baseline: 3.49 ± 1.9; day 1: 7.95 ± 3.28; day 10: 8.28 ± 3.07) and mixed apneas (baseline: 2.56 ± 1.00; day 1: 8.33 ± 2.38 apneas/h; day 10: 11.49 ± 4.60 apneas/h) compared with baseline contributed to the alteration in the apnea ratio in the experimental group (P = 0.03; Table 2).

The apnea/hypopnea indexes measured from the experimental group on day 1 were significantly correlated (r = 0.78; P = 0.002) to the HVR that was determined from the last two episodes of the intermittent hypoxia protocol presented on that day (Fig. 3). A similar relationship was also evident on day 10. In contrast, an inverse correlation between the apnea/hypopnea indexes and the magnitude of vLTF measured on day 1 was not evident (r = 0.38; P = 0.20; Fig. 3). The relationship was also absent on day 10. The apnea/hypopnea indexes measured from the control group on day 1 or 10 were not correlated to the ventilatory response measured during the last two episodes of the sham protocol (P ≥ 0.52) or to the magnitude of minute ventilation during the end-recovery period (P ≥ 0.39).

The average ΔPes during apneas and the average tidal volume measured from the initial three breaths following apnea events provided additional evidence that enhancement of the HVR may have contributed to the increase in apnea number on day 1 and 10 (Table 3). More specifically, ΔPes increased on day 1 and 10 compared with baseline (P = 0.03; Table 3). Moreover, this relationship remained when ΔPes/ΔSaO2 was compared between baseline and day 1 and 10 (P = 0.02; Table 3). Nonetheless, the increase in ΔPes or ΔPes/ΔSaO2 should be viewed with some caution since the differences noted between baseline and day 1 and 10 were eliminated after ΔPes was standardized for apnea duration (ΔPes/Δt; Table 3). This latter
finding could indicate that some other time sensitive stimulus impacted on $\Delta$Pes. Nevertheless, in support of the Pes measures, the average tidal volume following apneic events on day 1 and 10 (i.e., $V_T$ after apnea; Table 3) was greater compared with the baseline sleep study in the experimental group ($P = 0.003$; Table 3). The increase in tidal volume was accompanied by decreases in $\text{PETCO}_2$ on day 1 and 10 compared with the baseline sleep study ($P = 0.01$; Table 3). In contrast, the increase in tidal volume and alterations in $\text{PETCO}_2$ following apneic events were similar on day 1 and 10 and during the baseline sleep study in the control group (Table 3).

During apneic events, the $\Delta R$-$R$ interval and $\Delta R$-$R$ interval-$\Delta t$ on day 1 and 10 were similar to the measures obtained during the baseline sleep study in both the experimental and control group (Table 3). In contrast, the average $R$-$R$ interval measured during the inspiratory phases of the three breaths that followed each apnea was significantly greater on day 1 and 10 compared with baseline in both the experimental ($P = 0.004$) and control groups ($P = 0.03$) (Table 3).

**DISCUSSION**

We found that the number and duration of apneic events increased following brief exposure to intermittent hypoxia and sustained hypercapnia (i.e., carbon dioxide was sustained 3 Torr above baseline). This increase may have been due to enhancement of the HVR. A reduction in apneic events over time was also revealed in some participants following repeated daily exposure to the stimuli, although the number of events remained above baseline values. Most surprisingly, we discovered that repeated daily exposure to slightly elevated levels of carbon dioxide (3 Torr above baseline) for a short period of time elicited a reduction in the number of breathing events on the final day of the protocol compared with baseline in the control group.

**Methodological Considerations**

In the present study, we exposed participants to intermittent hypoxia during wakefulness to determine if the initiation of various forms of respiratory plasticity impacted on breathing stability during sleep. The reasons for selecting the number and duration of hypoxic episodes, as well as the reasons for sustaining carbon dioxide levels 3 Torr above baseline levels during exposure to intermittent hypoxia while awake, has been outlined in detail in a previous publication (15).

The individuals that were recruited for our investigation served as an ideal model to study the relationship between intermittent hypoxia and breathing (in)stability in individuals with sleep apnea. Our participants were relatively young, did not suffer from other comorbid conditions (diabetes, cardiovascular disease, and obesity), and were typically exposed to mild hypoxemia at night. Although baseline apnea/hypopnea indexes were moderate in some participants, this value was likely magnified compared with clinical measures, since Pes and sensitive measures of airflow (i.e., pneumotachograph) were used in combination with a liberal scoring criteria (30% reduction in airflow accompanied by a 3% reduction in $\text{SaO}_2$) to identify obvious hypopneas. Given these characteristics, we believed that a brief exposure to a slightly more severe level of hypoxemia than normally experienced would elicit the desired initiation of progressive augmentation and long-term facilitation, while minimizing potential risks (e.g., elevations in blood pressure), which were monitored day to day and overseen by a data safety monitoring board. These forms of respiratory plasticity may not be initiated in individuals with more severe forms of sleep apnea (30).

The Pes and tidal volume measures that we obtained in the present investigation were used as indexes of respiratory drive during sleep. The results obtained using these methods are less specific compared with other methods used to determine the apneic threshold and the tidal volume-$\text{PETCO}_2$ relationship during sleep (7, 31, 57). We chose to utilize these measures to avoid any potential disruption to sleep that are associated with these other methodologies, since our investigation was a first step toward determining if the initiation of respiratory plasticity impacts on breathing events during sleep.

**Outcome Following a Single Exposure to Intermittent Hypoxia**

We reported previously that a short exposure (i.e., 48 min) to intermittent hypoxia during wakefulness induced progressive augmentation of the ventilatory response to hypoxia/hypercapnia and vLTF in humans that are healthy (17, 24, 29, 36) and in humans suffering from sleep apnea (15, 22, 24). Given these
findings we were interested in exploring the potential physiological significance of these phenomena in individuals exposed to intermittent hypoxia on a nightly basis (i.e., individuals suffering from sleep apnea). We and others (26, 30) have hypothesized that V̇LTF and long-term facilitation of upper airway muscle activity might serve to mitigate breathing events during sleep. Ventilatory long-term facilitation could serve to ensure that minute ventilation was sustained in the presence of hypopcapnia, thereby preventing centrally induced apnea and obstructive events that often ensue (30). Moreover, long-term facilitation might enhance the activity of upper airway muscle activity, which could serve to stabilize the upper airway during sleep (26). In contrast, progressive augmentation of the HVR could promote breathing events because it leads to a reduction in the carbon dioxide reserve, which promotes hypocapnia. The carbon dioxide reserve is defined as the difference between the partial pressure of carbon dioxide associated with breathing at rest and the partial pressure of carbon dioxide that demarcates the apneic threshold (i.e., the point at which breathing ceases in response to a reduction in carbon dioxide during sleep). A reduction in the carbon dioxide reserve is due to increases in the apneic threshold or the ventilatory response to carbon dioxide. Mechanistically both of these alterations lead to progressive augmentation (please see Ref. 11 for a detailed explanation regarding the relationship between the HVR and carbon dioxide). Thus progressive augmentation of the HVR may be inextricably linked to reductions in the carbon dioxide reserve. The role that progressive augmentation has in reducing the carbon dioxide reserve is supported by the findings of Chowdhuri et al. (7) who showed that exposure to intermittent hypoxia ultimately leads to decreases in the carbon dioxide reserve via enhancement of the ventilatory response to carbon dioxide in healthy humans following exposure to intermittent hypoxia. Moreover, Salloum et al. (47) showed that the carbon dioxide reserve and ventilatory sensitivity to carbon dioxide was increased in humans with sleep apnea. These alterations could be the result of nightly exposure to intermittent hypoxia.

In our study, the initiation of progressive augmentation during wakefulness appeared to have impacted on breathing stability during sleep, since the number of nocturnal breathing events increased following exposure to intermittent hypoxia. This suggestion is supported by our findings that revealed that the HVR measured from the last two episodes of the intermittent hypoxia protocol was correlated to the apnea/hypopnea index. Moreover, ΔPes, which was employed as a secondary measure of respiratory drive during apneic events, increased following exposure to intermittent hypoxia. This enhancement was not evident following exposure to the sham protocol. In addition, the amplitude of the tidal volume response immediately following apneic events was also enhanced in the experimental group but not the control group. Interestingly, the increase in ΔPes and the amplitude of the tidal volume response did not appear to be due to differences in hypoxic intensity during apneic events across trials, since ΔPes/ΔSaO₂ was enhanced and the minimum SaO₂ was similar during apneic events on day 1 and 10 compared with baseline. Collectively, our results indicate the strong possibility that an increase in respiratory drive, possibly induced by progressive augmentation of the HVR (i.e., by alterations in chemoreflex sensitivity or the apneic threshold), may have been responsible for the increase in the number of breathing events that was observed.

Despite these findings, it is possible that other mechanisms could have been responsible for the increase in the apnea/hypopnea index that was observed (27). One alternative is that decreases in the arousal threshold resulted in the enhancement of the respiratory drive independent of alterations in chemoreflex sensitivity. However, our measures of ΔEKG during apneic events and the minimum R-R interval during the inspiratory phase of three breaths following events indicated otherwise, since no significant difference in ΔEKG during apneic events was evident on day 1 and 10 compared with baseline in either the experimental or control group. Likewise, the minimum R-R interval during the inspiratory phase of the three
breaths that followed the apneic events was longer on day 1 and 10 compared with baseline, which is contrary to the shortening that was expected. Lastly, apnea duration increased on day 1 and 10 compared with baseline in the experimental group, which is contrary to the reduction that might be expected if the arousal threshold was reduced.

Another alternative is that increases in the apnea/hypopnea index were principally a consequence of hypoxic depression of the central nervous system that led to blunted protective responses of upper airway muscles. This phenomenon has been observed in healthy individuals exposed to short periods of sustained hypoxia (12) but was not evident in individuals with obstructive sleep apnea following exposure to a similar stimulus under conditions of resistive loading (19). Consequently, the impact of this mechanism on the induction of apnea remains uncertain. Nevertheless, this mechanism could have a significant role in the induction of apnea in individuals chronically exposed to sustained moderate/severe hypoxia for long periods of time. However, it is less likely that this mechanism was principally responsible for the increase in the apnea/hypopnea index that we observed. Acute exposure to mild intermittent hypoxia for a short duration of time typically leads to enhancement of electromyographic activity of the genioglossus muscle in rats (46) and humans (6, 17). Moreover, the decrease in the apneic/hypopnea index that was evident from day 1 to 10 (see Outcome Following Repeated Daily Exposure to Intermittent Hypoxia for further discussion) in some of our participants would not be expected if the primary outcome of exposure to the intermittent hypoxia protocol was a blunted protective response.

Our findings also suggest that initiation of vLTF following exposure to acute intermittent hypoxia during wakefulness did little to mitigate breathing events during sleep immediately following exposure. The suggestion is supported by the absence of a correlation between the magnitude of vLTF on day 1 and 10 and the apnea/hypopnea index. It is possible that the transition from wakefulness to sleep resulted in the attenuation or abolition of vLTF. This hypothesis is supported by our preliminary findings that indicate that the magnitude of vLTF is greater during wakefulness compared with sleep in humans (51) but is contrary to findings in rats that showed that the magnitude of vLTF is greater during sleep compared with wakefulness (37, 52). Likewise, our finding that vLTF did not impact on the number of breathing events during sleep appears to be in line with clinical studies that have reported that apnea severity increases across the night independent of sleep architecture (4, 5, 13, 49). One would anticipate that breathing events within a given stage of sleep would decline throughout the night if vLTF or long-term facilitation of upper airway muscle activity was initiated by nocturnal exposure to intermittent hypoxia. Nonetheless, even though vLTF did not appear to impact breathing events, after a one-time exposure to intermittent hypoxia, it does not preclude the possibility that this phenomenon might have a significant impact under different experimental conditions. Our previous work (17) has indicated that the manifestation of vLTF during wakefulness is dependent on the maintenance of carbon dioxide levels above baseline. In the present investigation, this condition was not maintained during sleep, which is one possible reason that vLTF did not impact on breathing events.

**Outcome Following Repeated Daily Exposure to Intermittent Hypoxia**

Following repeated daily exposure to intermittent hypoxia, the number of nocturnal breathing events remained above baseline levels on day 10. However, we also found that the number of breathing events was fewer on day 10 compared with day 1 in some of the participants (i.e., 8 of the 13 participants). It is possible that the decline simply represents variability in the measurement of the apnea/hypopnea index. However, given the results obtained from the control group (see below regarding a discussion of the findings from the control group), this may not be the case. The decrease could be related to an improvement in sleep architecture, accompanied by an increased arousal threshold from day 1 to 10 that ultimately contributed to breathing stability (55). However, sleep architecture and other measures of arousal (i.e., ΔEKG during apneic events) were similar on both days. Alternatively, the decrease could be related directly to repeated daily exposure to intermittent hypoxia, since the reduction observed is similar to observations made in healthy individuals (44, 56), and in individuals suffering from obstructive sleep apnea (23, 38), who were exposed to sustained hypoxia for 4–7 days while sojourning to high altitude. More specifically, nocturnal central (44, 56) and obstructive (23, 38) breathing events increased above baseline upon initial exposure to hypoxia at high altitude. Thereafter, the number of events declined but remained above baseline for the duration of the data collection period (38, 44, 56).

Daily exposure to intermittent hypoxia may have resulted in a decrease in the apnea/hypopnea index by initiating a widening of the carbon dioxide reserve without accompanying changes in the hypoxic ventilatory response. This widening tends to promote breathing stability since a greater decrease in carbon dioxide would be required to exceed the apneic threshold and initiate an apneic event (10). This suggestion is supported by the findings of Katayama et al. (21) who reported that the carbon dioxide reserve widened in dogs exposed to intermittent hypoxia 7–8 h/day over a period of 3 wk even though no initiation of vLTF was evident. Whether or not brief exposure to intermittent hypoxia on a daily basis, which was the model employed in our study, leads to similar alterations to those observed in dogs after chronic exposure to longer periods (7–8 h/day) of intermittent hypoxia (21) remains to be determined.

Alternatively, daily exposure to a mild increase in carbon dioxide, which we sustained throughout the intermittent hypoxia protocol, might also impact on mechanisms (e.g., carbon dioxide reserve) that ultimately improve breathing stability. This suggestion is supported by our results that showed that this stimulus resulted in a decrease in breathing events compared with baseline measures in the control group. This finding was unexpected, and additional studies are required to determine the potential mechanisms responsible for this observation. Nonetheless, published findings have reported that continuous exposure to sustained mild hypcapnia for 5 or more days resulted in increases in resting levels of carbon dioxide and decreases in the ventilatory response to carbon dioxide (8, 48). This decrease could be a consequence of a reduction in chemoaux sensitivity, which could lead to the widening of the carbon dioxide reserve and promote breathing stability.
Given these findings, a reduction in ΔPes or increases in baseline levels of carbon dioxide might be expected on day 10 compared with day 1; particularly in the control group since the reduction in the apnea/hypopnea index on day 10 was below baseline and was evident in all but two participants. However, this was not the case. The lack of a reduction in ΔPes could be due to the insensitivity of the measure we employed. Consequently, more refined measures are likely required to address if chemoreflex sensitivity to carbon dioxide is reduced following repeated daily exposure to sustained hypercapnia. Likewise, long stable periods of baseline breathing during sleep in our participants were absent because of the presence of repetitive breathing events. This difficulty might have lead to our inability to detect small but significant differences in baseline measures of carbon dioxide on day 1 compared with day 10.

Conclusions and Speculations

Progressive augmentation of the ventilatory response to naturally occurring intermittent hypoxia, or possibly other stimuli presented during apnea, might promote breathing instability in individuals with sleep apnea. Findings from the present investigation support the hypothesis since enhancement of the ventilatory response to hypoxia, or other stimuli presented during apnea, might wholly or in part be responsible for the progressive increase in apnea that has been previously reported in clinical investigations (4, 5, 13, 49). We also hypothesized that vLTF might mitigate breathing stability, but it was unclear whether the initiation of this phenomenon could diminish or eliminate the potential influence of progressive augmentation on breathing stability. Under the experimental conditions employed vLTF initiated during wakefulness did not impact on breathing stability during sleep. This finding implies that under naturally occurring circumstances vLTF may not be maintained because of prevailing conditions that include arousal state (wake vs. sleep) and carbon dioxide levels (i.e., hypocapnia).

Lastly, independent of whether or not breathing stability was promoted or mitigated on day 1, we anticipated that the changes would be further magnified on the last day of the protocol if progressive augmentation and/or long-term facilitation were enhanced compared with the initial day of the protocol. Given that the number of events increased following acute exposure to intermittent hypoxia on day 1, we anticipated that this increase would be further magnified on the final day of the protocol. However, the number of breathing events decreased in some participants on the final day compared with the initial day of the protocol. Our results indicate that exposure to sustained mild hypercapnia could be responsible for this decline, although repeated exposure to intermittent hypoxia could not be ruled out. This finding is exciting since it reveals that the impact of exposure to a given stimulus, whether it is brief periods of intermittent hypoxia or sustained mild hypercapnia, may vary across time domains. For example, although an acute exposure to intermittent hypoxia might initiate forms of respiratory plasticity that lead to an increase in breathing events, repeated exposure may lead to subsequent alterations in respiratory motor control that lead to a diminution in breathing instability. From a more practical point of view, our findings suggest that brief daily exposure to sustained mild hypercapnia could have potentially beneficial effects on breathing stability during sleep and consequently could be used as an adjunct therapy in some individuals suffering from sleep apnea.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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