Effect of Hypercapnia on Sleep and Breathing in Unanesthetized Cats

Jimmy J. Fraigne, MS; Witali L. Dunin-Barkowski, PhD; John M. Orem, PhD

1Texas Tech University Health Sciences Center School of Medicine, Department of Cell Physiology and Molecular Biophysics, Lubbock, TX; 2Department of Neuro-Optical Systems, Scientific Research Institute for System Analysis, Russian Academy of Science, Moscow, Russia; 1IUP Génie Physiologique Informatique, Université de Poitiers, Poitiers, France

Objectives: In this study, we looked at the effect of hypercapnia on sleep architecture and breathing. We characterized the effect of hypercapnia on duration, frequency, and latency of NREM and REM sleep. We described state-specific patterns of breathing as well. This study is relevant to understand possible treatments for sleep disordered breathing.

Methods: Four cats were studied during 3-hour sessions while breathing 0%, 2%, 4%, and 6% CO₂ in room air. Each animal was studied 4 days per week for a period of 4 weeks. The animals breathed through a tube inserted into the trachea via a surgically created fistula. Respiration was measured using pneumotachography, and brain activity was recorded from implanted electrodes to discriminate states of sleep and wakefulness.

Results: Two percent inspired CO₂ increased sleep duration and decreased time awake. On the other hand, 6% CO₂ induced a worsening of sleep parameters: the duration of wakefulness increased by 24.2%. As a response to hypercapnia, tidal volume (Vt), minute ventilation (Ve), and respiratory effort (Vt/T) increased proportionally in all states with increasing levels of CO₂. With 6% CO₂ breathing tended to become similar in all states of consciousness. All breathing parameters converged towards a common value independently of the states.

Conclusion: We conclude that a mild hypercapnic stimulus can stimulate both breathing and sleep, and it may be useful in treatment of sleep disordered breathing.

Keywords: sleep architecture, carbon dioxide, rapid eye movement sleep, ventilatory response, chemosensitivity.

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FEW STUDIES HAVE LOOKED AT THE EFFECT OF CARBON DIOXIDE ON SLEEP ARCHITECTURE. WE FOUND THAT LOW LEVELS OF carbon dioxide (hypocapnia) disrupted sleep, and in particular, reduced the amount of REM sleep. In that study, low oxygen concentrations were given to cats to simulate high altitude. Ventilation increased in response to the hypoxia and caused a reduction in the level of carbon dioxide in the animal’s blood. Sleep was correspondingly disturbed, but sleep improved when carbon dioxide levels were increased to normal even though oxygen was kept low. This suggested that hypocapnia rather than hypoxia was the cause of disturbed sleep at altitude—a suggestion supported by results showing that REM sleep was depressed in a dose-dependent manner by the depth of hypocapnia produced by mechanical hyperventilation.

A recent study in patients with sleep disordered breathing has shown that use of carbon dioxide in combination with continuous positive airway pressure is effective for treatment of mixed central and obstructive sleep apnea. In these patients the respiratory disturbance index improved and arousal events decreased with addition of 0.5% to 1.5% CO₂ to the inspired air. However, the effect of higher than normal levels of carbon dioxide on sleep architecture in these patients, or in normal subjects, is not known.

Thus, we sought to determine the effect of mild and severe hypercapnia on sleep architecture. Furthermore, we wanted to determine the effect of hypercapnia on breathing during sleep and wakefulness. To test this, we analyzed duration, frequency, and latency of NREM and REM sleep, as well as tidal volume, frequency, minute ventilation, and effort of breathing when subjects (unanesthetized adult cats) breathed 0%, 2%, 4%, and 6% CO₂.

MATERIALS AND METHODS

Subjects

Four adult cats (3.9–5.0 kg) were prepared for recordings of electroencephalographic (EEG), pontogeniculo-occipital (PGO), and diaphragmatic electromyographic (EMG) activity. Tracheal fistulas were created, and headcaps containing a connector for EEG, PGO, and EMG electrodes were attached to the animals’ skulls. The headcap also contained standoffs that were used to immobilize the animal’s head during recordings. The animals recovered from surgery for one month before experimentation. The Animal Care and Use Committee of Texas Tech University Health Sciences Center School of Medicine approved all surgical and experimental procedures.

Surgical Procedures

The animals were anesthetized with acepromazine maleate (2.5 mg, i.m.) and ketamine (17 mg/kg, i.m.). A chronic tracheal fistula was created as described in a previous publication, and a tube was inserted through the fistula into the trachea. Anesthesia was maintained by administration of 1%-2% halothane in O₂.

The animals were instrumented also for recording of diaphragmatic activity, but these recording were lost in some cas-
es over the long duration of these experiments; consequently, diaphragmatic activity was not analyzed in the current study.

The animal was placed in a stereotaxic frame, and a midline incision exposed the dorsal skull. Four EEG electrodes (4-40 stainless steel screws with multistranded Cooner (AS 632) stainless steel leads) were screwed into the skull bilaterally over medial occipital and parietal cortices. Tripolar electrodes were implanted bilaterally at coordinates A6.4, L10 and H+2.5 in order to record PGO waves. EEG and PGO electrodes, and 4-40 anchor screws were cemented to the skull. A prefabricated headcap containing standoffs for immobilization of the head was fixed to the skull with dental cement. Gold Cinch pins were crimped to the ends of the diaphragmatic, EEG and PGO electrode wires and were inserted into a connector block. The connector block was then attached to the headcap.

Recording Procedures and Experimental Protocol

To reduce the amount the animals slept on nights before recording sessions, the animals were housed from 17:00 to 08:00 in a cold (0°C) environment.

After removal from the cold environment and a short period (~1 h) for feeding and exercise, the animal was placed in a veterinary bag, and its head was restrained by attachment of the standoffs of the headcap to the apparatus. The connector block was connected to amplifiers and to the digital recording system.

The trachea was intubated with a 4.0 mm inside diameter endotracheal tube that was attached to a Validyne pneumotachograph. Total dead space of the tracheal tube and pneumotachograph was 8 mL, which is approximately equal to the dead space of the upper airway. Tidal CO₂ levels were measured with an infrared CO₂ analyzer (GEMINI respiratory gas analyzer). Tidal CO₂ percentages, EEG, and PGO activity, and airflow were recorded on computer with the CED spike2 v 4.10 software from Cambridge Electronic Design. Signals were amplified by a P511 AC amplifier (Astromed). To administer CO₂, a 3-way plastic tube was attached to the endotracheal tube. One arm of the tube was attached to the CO₂ source, the second arm was attached to the pneumotachograph, and the third arm served as a flow-by exhaust for the CO₂ and for the animal’s expired gases. The length of this arm was adjusted to prevent inspiration of room air. We mixed atmospheric gas and carbon dioxide from a tank of 30% CO₂ and 70% N₂ to obtain the desired carbon dioxide concentration in the inspired air.

The day to day sequence of the different experimental conditions was elaborated using a 4x4 Latin square design. The treatments were a control treatment (room air) and 2%, 4%, and 6% carbon dioxide in room air. The 3-h experiments were conducted during 4 days on 4 different weeks, with one treatment condition per session for each cat. Thus, each of the 4 conditions was studied 4 times over a period of 4 weeks with variations in the day of the week to counteract an order effect.

Figure 1—Continuous record of airflow (inspiration up), tidal CO₂, EEG, and PGO waves during light NREM (LNREM), deep NREM (DNREM), and REM sleep in normocapnic conditions (0% CO₂). As in most cases, DNREM occurred as a state that heralded REM sleep.

Figure 2—Accuracy of visual sleep scoring in comparison to spectral analysis in one animal (BE4). A: Hypnogram in hypercapnia (2% CO₂), DNREM = deep NREM, LNREM = light NREM; B: Delta power as a function of time for the same session as in A; C: Power spectrum of various frequency bands (absolute values) as a function of time for the same session as in A.
On average, the end-tidal CO\textsubscript{2} during NREM sleep was brought to 34.6, 37.0, and 45.1 mm Hg with 2%, 4%, and 6% inspired CO\textsubscript{2} respectively, and 32.7 mm Hg with just room air.

A carbon dioxide calibration was obtained at the beginning and end of each 3 h experiment. For this we used a gas sample known to have a concentration of 5% CO\textsubscript{2}. A calibration of the airflow signal was also done with the use of a ventilator. A volume of 50 mL was delivered to the pneumotachograph at 3 different rates at the end of the experiment. This calibration allowed us to calculate tidal volumes from the airflow signals.

The decreased partial pressure of oxygen in the inspired air (P\textsubscript{I}O\textsubscript{2}) resulting from addition of carbon dioxide into room air did not decrease the partial pressure of oxygen in the alveoli (P\textsubscript{A}O\textsubscript{2}). We confirmed this assumption by using the alveolar-gas equation as described below:

\[
P\textsubscript{A}O\textsubscript{2} = P\textsubscript{I}O\textsubscript{2} - \left( \frac{V\text{O}_2}{V\text{A}} \right) \times k
\]

With P\textsubscript{A}O\textsubscript{2}: the partial pressure of oxygen in the alveoli; P\textsubscript{I}O\textsubscript{2}: the partial pressure of oxygen in the inspired air; V\text{O}_2: the oxygen consumption; V\text{A}: The alveolar ventilation; and k: a constant with a value of 863.

We obtained P\textsubscript{I}O\textsubscript{2} via the following equation:

\[
P\textsubscript{I}O\textsubscript{2} = F\text{I}O\textsubscript{2} \times \left[ P\text{B} - P\text{H}_2O \right]
\]

With F\text{I}O\textsubscript{2}: the fraction of oxygen in the inspired gas; P\text{B}: the barometric pressure; and P\text{H}_2O: the water vapor pressure (47 mm Hg).

Using these parameters, we obtained P\textsubscript{A}O\textsubscript{2} values ranging from 86.3 mm Hg for 2% CO\textsubscript{2} to 80.8 mm Hg for 6% CO\textsubscript{2}. The P\textsubscript{A}O\textsubscript{2} for control conditions (0% CO\textsubscript{2}) was 89.7 mm Hg. Despite a minor right shift of the oxygen dissociation curve with increased P\textsubscript{CO2}, these P\textsubscript{A}O\textsubscript{2} values are positioned on the plateau phase of the oxygen dissociation curve (i.e., in the 90%-100% hemoglobin saturation range). Hence, a hypoxic stimulus was not generated with our experimental conditions.

During each experiment, the cat was left alone and the recorded parameters were monitored in another lab to prevent interaction and artifact due to the presence of the experimenter. Recordings lasted no more than 3 h and typically occurred from 09:00 to 12:00. The animal was allowed unrestricted exercise and ad lib access to food and water in the laboratory from the end of the recording until 17:00.

Sleep Data Analysis

NREM and REM sleep and wakefulness were defined on the basis of standard EEG criteria. NREM sleep was divided into 2 stages, light NREM sleep (LNREM) and deep NREM sleep (DNREM). LNREM was scored as such if the characteristics (δ activity ([0.5-4 Hz]) of NREM sleep were sustained for more than 50% of an epoch (28 s) without PGO wave inclusions (Fig 1). We identified DNREM as such if the characteristics of NREM sleep were sustained for more than 75% of an epoch (28 s) with inclusion of isolated PGO wave activity (Fig 1). The sleep scoring was done visually by epoch of 28 seconds for each recording session. Then, the collected information was compiled to determine a hypnogram, the duration of sleep and of its different stages, the frequency of sleep cycles and phases, and the sleep latency. For each REM period, PGO density (number of PGO waves per minute) was analyzed.

To further verify that our visual scoring was appropriate, in one animal we computed EEG frequency bands using a spectral analysis program designed to execute a fast Fourier transform on 28-s epochs with 1020 sampling points (Spike2 and Matlab software). We defined frequency bands as: delta (δ) 0.5-4 Hz, theta (θ) 5-8 Hz, alpha (α) 9-14 Hz, and beta (β) 15-40 Hz (Fig 2).

Breathing Analysis

A breath-by-breath analysis of respiratory parameters was written using Spike2 software. Tidal volume (V\textsubscript{T}), duration of
Hypercapnia Modified Sleep Architecture

Hypercapnia had mixed effects on sleep (Fig 3). A low level of carbon dioxide added to the inspired air stimulated sleep. On the other hand, a high concentration of carbon dioxide was a strong arousing stimulus, as reported previously.1,2

**Effect of 2% Carbon Dioxide on Sleep**

Administration of 2% carbon dioxide increased total sleep duration by 21.0% and NREM sleep by 25.4% (DNREM sleep by 24.5% and LNREM sleep by 26.0%) compared to control values (Figure 4, Table 1). This increase of sleep duration was due mainly to a 22.7% increase in the average duration of NREM sleep periods compared to the normocapnic condition, rather than an increase in the number of episodes. The number of DNREM and REM periods increased by 18.9% and 12.5%, respectively, compared with control, but the increases were not significant statistically. Sleep latency, the time to the first sleep stage (in all cases LNREM sleep), was decreased by 39.1% compared to control. Finally, wakefulness duration was significantly decreased by 16.2%.

**Effect of 4% Carbon Dioxide on Sleep**

A level of 4% inspired CO₂ did not significantly disturb sleep (Figure 4 and Table 1). Four percent CO₂ led to a slight but not significant decrease in DNREM and REM duration, but it did not alter the overall duration of NREM sleep. This decrease in DNREM and REM sleep duration was due mainly to a decrease in number of occurrences. However, total sleep duration, NREM sleep duration, and number of NREM periods were practically unchanged.
Effect of 6% Carbon Dioxide on Sleep

Addition of 6% CO₂ to the inspired air led to a 41.2% decrease in total sleep duration. This decrease was due to a significant decrease of REM, NREM and DNREM duration as well as a decrease in the number of REM and DNREM periods. We observed 33.9% and 68.6% decreases in NREM and REM sleep durations, respectively; and decreases of 39.6% and 62.5% in the number of DNREM and REM periods, respectively. LNREM duration, LNREM occurrences, and NREM occurrences were not significantly affected by 6% CO₂ but tended to decrease. The significant decrease of NREM duration can be explained by a 30.2% decrease in the duration of NREM episodes. Sleep latency was increased by 20.3% compared to control, and wake duration was significantly increased by 23.6%, demonstrating a stimulating effect of 6% carbon dioxide (Figure 4, Table 1).

The enhancement of sleep with addition of 2% CO₂ in the inspired air, and the worsening of sleep parameters with 6% CO₂, could be seen across the 3-h period. Overall sleep and wakefulness parameters were modified, for each cat, in the same manner as illustrated for individual data (Fig 5). Moreover, variation of frequency band intensity as a function of time matched the sleep cycles of a given hypnogram (Fig 2).

REM Sleep During Hypercapnia

As shown in Figure 6, the variability of tidal volume during REM sleep decreased proportionally with increasing levels of carbon dioxide. Breathing became more regular when carbon dioxide was added to inspired air. We observed 19.6%, 53.3%, and 67.9% decreases in breathing variability with 2%, 4% and 6% CO₂, respectively, compared with control. PGO density was not significantly affected by the various conditions but tended to increase with increasing carbon dioxide level.

Hypercapnia Induced an Increase in Ventilation During Wakefulness and Sleep

The ventilatory response during each sleep state and wakefulness are presented in Figure 7. The results described here are weighted averages of numerous breaths during each recording session, for all given conditions and for each state of consciousness independently.

Minute ventilation (Vₑ) increased with increasing levels of carbon dioxide because of a large increase in tidal volume. Increased Vₑ, Vₑ, and effort (Vₑ/Tₑ) with increasing CO₂ level were observed in each of wakefulness, NREM, and REM sleep. All three parameters were significantly higher during NREM sleep than in other states for all conditions. Moreover, Vₑ and
Hypercapnia induced hyperventilation in unanesthetized adult cat. This respiratory response was mainly the result of an increased tidal volume that was proportional to CO₂ level. Total duration of the breathing cycle increased with a mild hypercapnic stimulus and tended to decrease toward control values with higher levels of carbon dioxide. Thus, each breath during hypercapnia had greater amplitude and a longer or similar duration compared to control.

**DISCUSSION**

Hypercapnia induced hyperventilation in unanesthetized adult cat. This respiratory response was mainly the result of an increased tidal volume that was proportional to CO₂ level. Total duration of the breathing cycle increased with a mild hypercapnic stimulus and tended to decrease toward control values with higher levels of carbon dioxide. Thus, each breath during hypercapnia had greater amplitude and a longer or similar duration compared to control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2% CO₂</th>
<th>4% CO₂</th>
<th>6% CO₂</th>
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<tr>
<td>TIA</td>
<td>178.2 (1.8)</td>
<td>176.9 (3.2)</td>
<td>175.8 (2.0)</td>
<td>172.6 (3.5)</td>
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<td>Wₜ</td>
<td>104.6 (5.3)</td>
<td>87.7 (6.2)*</td>
<td>110.9 (6.5)</td>
<td>129.3 (6.4)*</td>
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<td>SleepₜTOT</td>
<td>73.6 (6.1)</td>
<td>89.1 (6.3)*</td>
<td>65.0 (5.5)</td>
<td>43.3 (4.6)*</td>
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<tr>
<td>NREMₜ</td>
<td>58.2 (6.5)</td>
<td>73.0 (6.2)*</td>
<td>53.6 (4.5)</td>
<td>38.5 (4.7)*</td>
</tr>
<tr>
<td>LNREMₜ</td>
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<td>45.6 (4.5)</td>
<td>38.3 (3.5)</td>
<td>29.3 (3.9)</td>
</tr>
<tr>
<td>DNREMₜ</td>
<td>22.0 (3.8)</td>
<td>27.4 (3.8)</td>
<td>15.2 (2.8)</td>
<td>8.9 (1.3)*</td>
</tr>
<tr>
<td>REMₜ</td>
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<td>16.1 (2.8)</td>
<td>11.4 (2.3)</td>
<td>4.8 (1.6)*</td>
</tr>
<tr>
<td>NREMₚ</td>
<td>33.9 (3.6)</td>
<td>34.6 (3.2)</td>
<td>35.9 (3.4)</td>
<td>32.1 (4.4)</td>
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<tr>
<td>LNREMₚ</td>
<td>35.5 (3.5)</td>
<td>38.0 (3.5)</td>
<td>36.9 (3.2)</td>
<td>31.2 (4.0)</td>
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<td>15.9 (2.3)</td>
<td>18.9 (2.4)</td>
<td>12.2 (1.4)</td>
<td>9.6 (1.5)*</td>
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<td>2.2 (0.4)</td>
<td>1.2 (0.4)*</td>
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<td>SLEEPₜ</td>
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<td>3.9 (1.1)</td>
<td>4.5 (0.9)</td>
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</table>

Data are means (SEM) based on 4 recording sessions for each animal. All values are time (T) in minutes, or number of periods (P), or latency time in minutes (L). TIA refers to time in apparatus in minutes, and SleepₜTOT to total sleep time during recording session. NREM sleep periods (NREMₜ) were defined as consecutive LNREM and/or DNREM sleep periods bounded either by wakefulness or REM sleep. LNREM sleep periods (LNREMₜ) were defined as consecutive NREM sleep periods bounded by any other states. DNREM sleep periods (DNREMₜ) were defined as consecutive DNREM sleep periods bounded by any other states. Hence, NREMₚ is not equal to the sum of LNREMₜ and DNREMₜ. *P < 0.05 compared to control.

Sleep architecture was affected also by carbon dioxide. Addition of 2% CO₂ increased total sleep duration by increasing NREM sleep time. Sleep latency tended to decrease with mild hypercapnia. On the other hand, further increases of CO₂ levels stimulated both respiration and arousal. Sleep parameters deteriorated with an inspired CO₂ of 6%. Surprisingly, the addition of 4% CO₂ in the inspired air did not produce any significant change in sleep duration compared to control.

**Respiratory Response to CO₂ and State Specific Patterns of Breathing During Hypercapnia**

Our subjects showed an increase in ventilation due to a larger Vₜ in all states and longer breath duration. Also, we observed that breathing tended towards a common value with increasing CO₂ in all states.

Bonora et al. showed that the increase in ventilation in response to CO₂ in adult cats was due mainly to an increase in Vₜ and not to an increase in frequency. Our results support their earlier report.

In our study, Vₜ and Vₑ were higher during NREM sleep than during other states. This result is surprising to us since it is thought that chemosensitivity is decreased during sleep compared to wakefulness. Breathing in NREM sleep is principally driven by chemical stimuli. In normal subjects, breathing in NREM sleep is described by an increase in tidal volume and a decrease in frequency compared to wakefulness, whereas, REM sleep and wakefulness present a greater frequency due to state-specific nonrespiratory inputs. Therefore, we think that an increased CO₂ drive enhances NREM sleep characteristics.
such as a greater V̇ and lowers breathing frequency, and leads to a greater ventilatory response. Horner et al.\textsuperscript{14} showed that ventilation as represented by diaphragmatic activity increases linearly with increasing CO\textsubscript{2} levels in inspired air (1% to 9% in their study). Also, they demonstrated that the genioglossus and diaphragm responded differently to hypercapnia, and they concluded that in NREM sleep and REM sleep genioglossal response to carbon dioxide was decreased and eliminated respectively. In our study, the upper airways were bypassed, which decreases resistance.\textsuperscript{13} However, it seems unlikely that the greater minute ventilation and tidal volume seen during NREM sleep compared to wakefulness was solely due to the loss of upper airway resistance in our model. In patients with obstructive sleep apnea, loss of upper airway muscle tone during sleep is a major component of the pathophysiology of the disease. Horner et al.\textsuperscript{14} demonstrated that a low level of carbon dioxide, such as the one we used in the present study, did not stimulate genioglossal activity during NREM and REM sleep in rats. It is not known if this is true also for humans.

Schafer et al.\textsuperscript{33} showed in humans that CO\textsubscript{2} stimulated breathing less in REM sleep than in NREM sleep. We found that ventilation was strongly stimulated during hypercapnia in all states. We observed REM-specific respiratory changes\textsuperscript{26} in all conditions, but we did not see any amplification of those changes with hypercapnia. On the contrary, we observed a reduction in breathing irregularities concomitant with an increase in PGO density with increasing inspired CO\textsubscript{2}. With 6% CO\textsubscript{2} during REM sleep, breathing become deeper and extremely regular, that is, similar to breathing during NREM sleep. Furthermore, we observed that with the highest carbon dioxide stimulus used in our study, all breathing parameters tended towards a common value in all states. Evidently in this case chemical drive overpowers state-specific inputs to the respiratory system.

Low Levels of Carbon Dioxide in Inspired Air Stimulate Sleep

As shown by several studies,\textsuperscript{24} carbon dioxide increases ventilation and causes arousal. However, these studies did not look at the effect of hypercapnia on sleep architecture. Moreover, none of them used a concentration of carbon dioxide less than 5%. Ioffe et al.\textsuperscript{32} showed that hypercapnia altered the sleep state pattern in rats. Their study used CO\textsubscript{2} concentrations of 6%, 7%, and 8% and demonstrated a worsening of sleep parameters. Horner et al.\textsuperscript{14} showed that sleep-wake cycles were not affected by carbon dioxide concentration lower than 5%. Wakefulness duration was increased significantly by increasing carbon dioxide from 5% to 7%. However unlike our results, they did not report any significant stimulation of NREM sleep with mild hypercapnic stimuli (1%-3% CO\textsubscript{2}).

Some nuclei involved in arousal, e.g., locus coeruleus, raphe, and the tuberomammillary nucleus, are also chemosensitive,\textsuperscript{17,18,21,22,23,24} which indicates possible pathways by which CO\textsubscript{2} can influence sleep. Mitchell et al.\textsuperscript{13} and Mulkey et al.\textsuperscript{13} argue that different central chemosensitive areas have different thresholds to hypercapnia. Chemosensitive cells of the retrotrapezoid nucleus of the ventral medulla have a greater sensitivity than other areas. We speculate that these cells respond at a lower level of carbon dioxide and drive breathing to increase ventilation, whereas chemosensitive wakefulness centers respond to higher levels of hypercapnia. This could explain the lack of arousal seen with 4% CO\textsubscript{2}. The lack of arousal does not explain however, our findings that 2% carbon dioxide increases total sleep duration by increasing NREM sleep duration. A study by Guntal et al.\textsuperscript{11} supports our results. This study showed that humans placed in a closed environment (a submarine) with an ambient CO\textsubscript{2} of 0.7% to 1.2% did not have a reduction of sleep quality and showed instead an increase of slow wave sleep amounts as the duration of CO\textsubscript{2} exposure over days increased. Several studies\textsuperscript{7,9,10,28,29} have shown that responses to hypercapnic stimuli are mediated by adenosine triphosphate and adenosine, and that hypercapnia induces an increase of extracellular adenosine, a potent endogenous somnogen.\textsuperscript{2,4,12,30} Indeed, the progressive increase of adenosine-during the waking hours has been linked to sleep homeostasis. We propose that a mild hypercapnia will stimulate the release of adenosine and stimulate sleep via its effect on the basal forebrain\textsuperscript{4} or other adenosine-sensitive areas.\textsuperscript{12} Thus a low level of carbon dioxide will induce release of adenosine to stimulate sleep and at the same time will stimulate the chemosensitive cells of the retrotrapezoid nucleus, and induce an increase in ventilation.

Our study is the first to show that a mild hypercapnic stimulus can stimulate breathing and increase NREM sleep. As mentioned in the introduction, this finding can be of interest for improving mixed central and obstructive apnea treatments.\textsuperscript{8} As shown by Thomas et al.\textsuperscript{13} addition of low levels (0.5%-1.5%) of carbon dioxide is an effective treatment for such conditions. In their study, no adverse effects on overall sleep architecture were noted. Nevertheless, an increase in REM sleep was suggested, and arousal index dropped significantly from 32.3 to 4.5 per hour of sleep.

Effect of High CO\textsubscript{2} Level on Sleep Architecture

High levels of CO\textsubscript{2} increased arousal. Total sleep duration decreased 41% with 6% inspired CO\textsubscript{2} compared to control. An

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**Figure 8**—Schematic representation of the effect of carbon dioxide on total sleep duration. Total sleep duration is represented as a function of end-tidal CO\textsubscript{2}. The dotted line corresponds to our previous study on hypocapnia.\textsuperscript{26} Hashed line (end-tidal CO\textsubscript{2} > 7.2) shows assumed values from studies by others.
even greater effect was seen on REM sleep, with an important decrease in the number of REM periods and a decrease in the duration of these periods. The rat study of Ioffe et al. also showed an increase in arousal with CO₂, but their study demonstrated that total REM duration was unchanged during hypercapnia. This was the consequence of a 30% increase in the duration of each REM period that counterbalanced a decrease in the number of those periods. In contrast to these results, we found that REM sleep was decreased due to a decrease in both the number and duration of REM periods. Their study was carried out on adult rat, and differences in metabolic and physiologic responses to hypercapnia in rodents and cats could explain the different results. The effect of hypercapnia on rodent metabolism is unclear. Some report decreased metabolism as seen during hypoxia, whereas others report no change.

Arousal may result from stimulation of arousal centers sensitive to CO₂/H⁺ (see above). However, according to Berry et al., the arousing effect of hypercapnia may result from an increase of breathing effort in response to CO₂ and not from CO₂ itself. In their study, the effect of hypercapnia on the arousal response to airway occlusion in humans was the main focus. Their findings revealed that an increase of PₐCa₁ before airway occlusion shortens the time to arousal by increasing the initial occluded inspiratory effort and the rate of increase in effort, but it does not change the effort threshold at which arousal occurs. We found that effort (Vₐ/Tₐ) increases proportionally with CO₂ in all states, and we cannot confirm or refute their idea. However, in our study, a mild hypercapnic stimulus increased the effort index and did not cause arousal.

**Hypocapnia vs. Hypercapnia Effects on Sleep**

We demonstrated previously that hypocapnia disrupts sleep and reduces the amount of REM sleep. In that study, low oxygen concentrations were given to cats to simulate high altitude. Breathing frequency increased in response to hypoxia and caused a reduction in the level of carbon dioxide in the blood. Sleep duration was decreased in these conditions but could be improved by adding carbon dioxide while maintaining low levels of oxygen. This suggested that hypocapnia rather than hypoxia was the cause of disturbed sleep at altitude. In the same study, we showed that REM sleep was decreased linearly with decreasing carbon dioxide levels (from 85% to 65% of eupneic end-tidal CO₂) produced by mechanical hyperventilation. Figure 8 illustrates schematically those and the present results. Hypocapnia and hypercapnia (6% CO₂) both decrease total sleep duration. However, the mechanisms involved in these effects do not seem to be identical. In the former study, only REM sleep was significantly affected by low levels of CO₂, whereas, as shown in the present study, high levels of hypercapnia significantly decreased both NREM and REM sleep. Finally, we demonstrate in the present study that a mild hypercapnic stimulus increases significantly the amount of NREM sleep. We speculate that increased extracellular adenosine level during hypercapnia leads to our findings.

**Conclusion**

In this study, we demonstrated that carbon dioxide causes a linear increase in ventilation in all sleep states and wakefulness. We also established that the effect of hypercapnia on sleep is discontinuous with increasing levels of CO₂ (Fig 8). Hypercapnia and a high level of CO₂ lead to stimulation of arousal, whereas, a moderate CO₂ level will stimulate sleep. Our study is the first to show that a mild hypercapnic stimulus can stimulate simultaneously breathing and sleep.

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