Hypocapnia Decreases the Amount of Rapid Eye Movement Sleep in Cats

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Context: Sleep is disturbed at high altitudes. Low Po_2 levels at high altitude cause hyperventilation, which results in secondary hypocapnia (low $Paco_2$ levels). Thus, although sleep disruption at high altitudes is generally assumed to be caused by hypoxia, it may instead be the result of hypocapnia.

Objective: To determine whether hypocapnia disrupts sleep.

Methods: Four cats were studied for a total of 345 hours of sleep recordings. Two methods were used to test this idea. First we studied their sleep when the cats breathed oxygen concentrations (15% and 10%) equivalent to those at approximately 12,000 feet and 21,000 feet. Then we studied their sleep again in response to the same hypoxic stimuli but with CO_2 added to the inspirate to maintain normal CO_2 levels. Second, we used mechanical hyperventilation to vary the levels of CO_2 while maintaining normal O_2 levels.

Results: Hypoxia (10% O_2) decreased the amount of rapid eye movement sleep to about 20% of normal, and adding back CO_2 restored rapid eye movement sleep to approximately 70% of normal. Periodic breathing and apneas were not observed during hypoxia in sleep. When mechanical hyperventilation lowered the CO_2 to 85%, 75%, and 65% of normal, rapid eye movement sleep decreased progressively from a control level of 17% of total recording time to 12%, 7%, and 4%, respectively.

Conclusion: We conclude that hypocapnia rather than hypoxia may account for most of the sleep disturbance at high altitudes.

Key Words: hypoxia, non-rapid eye movement sleep, carbon dioxide

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INTRODUCTION

POOR SLEEP QUALITY IS COMMON IN HYPOXIC ENVIRONMENTS, SUCH AS AT HIGH ALTITUDE.[1-3] Hypoxia disrupts both rapid eye movement (REM) sleep and non-REM (NREM) sleep.[4-11] The exact mechanisms of hypoxia-induced sleep disruption are unknown, but sleep-disordered breathing (periodic breathing and apneas) and frequent arousals may be involved. Periodic breathing is a pattern of waxing and waning breaths and commonly occurs at high altitude during sleep.[4,10,12-14] The waning phase of periodic breathing often culminates in apnea and is associated with arousals or nighttime awakenings.[15] However, arousals and awakenings can occur in hypoxic environments independent of sleep-disordered breathing, and the resulting sleep fragmentation can adversely affect sleep quality.[4,10]

Whatever the mechanism, it is generally assumed that the hypoxia per se causes the sleep disruption. However, this assumption is unwarranted. Low O_2 levels increase ventilation, which causes a secondary decrease in arterial CO_2 levels (hypocapnia). Thus, hypocapnia rather than hypoxia may cause the sleep disruption. To test this idea, we used 2 approaches. First, we studied the sleep of subjects (adult cats) when they breathed 15% and 10% O_2 , which, in Lubbock, Texas, elevation 1000 m (3280 ft), are equivalent to breathing at altitudes of approximately 3600 m (11,800 ft) and 6400 m (21,100 ft). Then we studied the cats' sleep again in response to the same hypoxic stimuli but with CO_2 added to the inspirate to maintain normal arterial CO_2 levels. We reasoned that this would improve sleep if hypocapnia was responsible for the sleep disturbance. Second, in another set of experiments, we manipulated just the level of CO_2 while maintaining O_2 at normal levels. This was done using mechanical hyperventilation. When mechanically ventilated, the animals were hypocapnic (and therefore apneic) but normoxic. The level of hypocapnia was adjusted using a computer-controlled CO_2 injector, which maintained the CO_2 at 85%, 75% or 65% of the normal level in NREM sleep. The 65% level is approximately equal to the level of hypocapnia created when the animals spontaneously hyperventilate in response to 10% O_2. We predicted that hypocapnia would disrupt sleep in a dose-response manner.

Disclosure Statement
No significant financial interest/other relationship to disclose.

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METHODS

Subjects

Four adult cats (3.2 kg to 5.3 kg in weight) were prepared for recordings of the electroencephalogram (EEG), pontogeniculooccipital (PGO) waves, and electromyographic (EMG) activity of the diaphragm. Tracheal fistulas were created, and head caps containing a connector for electrodes were attached to the animals' skulls. The head caps also contained standoffs that were used to immobilize the animal's head during recordings. The animals recovered from the operation for 1 month before experimentation. After recovery, they were adapted to the experimental apparatus. Details of the techniques have been published.[16] The Animal Care and Use Committee of Texas Tech University School of Medicine approved all surgical and experimental procedures.

Recording Procedures

On the nights before recording sessions, the animals were housed in a cold (0°C) environment in order to consolidate sleep the following day. During recordings, the trachea was intubated with a 4.0 mm endotracheal tube that was attached to a Validyne pneumotachograph (Validyne Engineering Corporation, Northridge, CA, USA). Pressure levels in the tube were measured using a volumetric pressure transducer. Tidal O_2 and CO_2 were measured with an O_2 analyzer (Beckman OM-11) and infrared CO_2 analyzer (Beckman LB-2). Tidal O_2 and CO_2 percentages, along with amplified signals of EEG, EMG, PGO, airflow, and intratracheal

pressures were recorded on paper (Astro-Med 9500 – Astro-Med Incorporated, West Warwick, RI, USA) and on magnetic tape. Recording sessions lasted approximately 3 hours.

Experimental Conditions

All experiments were conducted in Lubbock, Texas (elevation 1000 m).

In the first series of studies, the level of inspired O_2 was varied, and the CO_2 was either held at control levels (isocapnic hypoxia) or allowed to fall as a result of the hyperventilation produced by hypoxia (hypocapnic hypoxia). When the CO_2 is held constant during hypoxia, ventilation is further increased, which increases the arterial O_2 levels. In order to avoid this compounding factor, we maintained the level of hypoxia by increasing the amount of N_2 in the inspired air so that the end-tidal partial pressure of O_2 ($PETO_2$), and, thus, the arterial PO_2 was the same in both hypocapnic and isocapnic conditions. Accordingly, there were 5 conditions in this series: normoxia control (21% O_2), 10% O_2 in N_2, $PETO_2$ at 10% O_2 with CO_2 added back (10% + CO_2), 15% O_2 in N_2, and $PETO_2$ at 15% O_2 with CO_2 added back (15% + CO_2). Average end-tidal CO_2 in normoxia during NREM sleep was 32.7 mm Hg (upper limit, 34.1 ± 0.1 mm Hg; lower limit, 30.9 ± 0.1 mm Hg). On average, hyperventilation in response to 15% O_2 and 10% O_2 reduced the end-tidal CO_2 to about 89% (29.1 mm Hg) and 67% (21.9 mm Hg) of the end-tidal levels in NREM sleep under normoxic conditions. Each animal was studied over a period of 5 weeks. The condition on a particular day was determined using a Latin square design. Thus, each of the 5 conditions was studied 5 times over a period of 5 weeks with variations in the day of the week to counteract an order effect.

In a second series of experiments, mechanical hyperventilation was used to produce varying degrees of hypocapnia while maintaining normal O_2 levels. A 2-position valve switched the animal from breathing room air to a ventilator that delivered a 50-mL tidal volume at a rate of 50 per minute. The end-tidal CO_2 levels ranged from 65% to 85% of the NREM eupneic level and were produced using computerized pulse-width modulation of a CO_2 injector. In this series of experiments, there were 4 conditions: control (spontaneously breathing), end-tidal CO_2 at 65% of the NREM level, end-tidal CO_2 at 75% of the NREM level, and end-tidal CO_2 at 85% of the NREM level. The average end-tidal CO_2 in NREM sleep during spontaneous breathing in these cats was 32.8 mm Hg (upper limit, 34.1 ± 0.1 mm Hg; lower limit, 30.9 ± 0.1 mm Hg). Thus, 65%, 75%, and 85% of the NREM eupneic level were approximately 21.3 mmHg, 24.6 mmHg, and 27.9 mmHg, respectively. At all of these levels of CO_2, the animals were apneic and made no attempts to breathe. In REM sleep, however, the respiratory muscles were intermittently activated,[16] which caused minor perturbations in flow produced by the ventilator. Each animal was studied over a period of 4 weeks. The condition on a given day was determined using a Latin square design. Thus, in this second study, 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.

Table 1—Wakefulness and Sleep in Normoxia, Hypocapnic and Isocapnic Hypoxia in individual subjects

		Normoxia		Hypoxia		
Animal	Sleep Parameter	21% O_2	15% O_2	15% O_2+CO_2	10% O_2	10% O_2+CO_2
EDAM	TIA	185 (2.8)	180.9 (2.7)	184.3 (2.8)	178.7 (2.2)	188.7 (2.5)
	W_T	114.5 (12.9)	97.7 (6.7)	97.3 (5.4)	120.3 (9.3)	99.2 (13.7)
	$NREM_T$	44.9 (8.6)	55.0 (6.3)	61.5 (6.3)	55.6 (7.8)	72.5 (13.0)
	REM_T	25.6 (5.5)	28.3 (2.5)	25.4 (3.8)	2.8 (2.7)	17.0 (3.6)
	REM_P	2.8 (0.7)	2.4 (0.3)	3.0 (0.4)	0.4 (0.3)	1.8 (0.2)
	REM_{LAT}	30 (7.7)	22.0 (3.6)	22.9 (13.2)	145.4 (26.1)	44.3 (14.9)
	$Sleep_{LAT}$	10 (5.1)	2.8 (1.0)	1.9 (1.3)	1.6 (0.0)	5.7 (2.0)
	$Sleep_{TOT}$	70.5 (13.7)	83.2 (6.8)	87.0 (6.0)	58.4 (8.4)	89.5 (14.3)
	SE	38 (7.1)	46.0 (3.7)	47.2 (3.0)	32.8 (4.8)	47.3 (7.3)
SUMR	TIA	177.7 (1.0)	174.7 (5.9)	171.1 (3.2)	187.5 (5.5)	182.7 (1.4)
	W_T	42.1 (10.9)	30.0 (14.3)	28.3 (6.1)	76.9 (18.8)	48.7 (17.0)
	$NREM_T$	101.4 (9.7)	103.8 (11.1)	110.3 (8.5)	100.5 (14.7)	111.2 (13.4)
	REM_T	34.2 (1.8)	41.0 (4.4)	32.6 (2.0)	10.0 (4.2)	22.9 (6.1)
	REM_P	5.8 (0.4)	5.4 (0.4)	5.5 (0.3)	1.6 (0.6)	4.4 (0.9)
	REM_{LAT}	26.4 (5.8)	10.2 (4.4)	27.9 (5.6)	93.1 (27.3)	25.7 (2.5)
	$Sleep_{LAT}$	5.7 (2.4)	2.2 (0.4)	0.4 (0.5)	8.8 (5.2)	0.0 (0.0)
	$Sleep_{TOT}$	135.6 (11.4)	144.8 (10.1)	142.9 (6.9)	110.5 (14.6)	134.1 (17.7)
	SE	76.3 (6.2)	83.5 (7.2)	83.5 (3.6)	59.6 (8.9)	73.2 (9.3)
CHDR	TIA	182.1 (1.2)	184.1 (5.1)	189.4 (4.2)	174.5 (8.1)	179.4 (3.5)
	W_T	115.5 (8.7)	109.9 (18.7)	109.5 (8.9)	105.0 (9.5)	117.0 (11.2)
	$NREM_T$	42.7 (7.3)	56.1 (12.6)	53.8 (9.2)	64.6 (7.1)	42.0 (9.3)
	REM_T	23.9 (2.8)	18.1 (2.2)	26.1 (4.5)	4.9 (2.0)	20.4 (2.2)
	REM_P	6 (0.6)	5.3 (1.0)	5.3 (0.6)	2.5 (1.0)	4.8 (0.6)
	REM_{LAT}	40.8 (9.5)	38.1 (9.5)	34.7 (9.0)	78.2 (41.9)	26.3 (4.9)
	$Sleep_{LAT}$	12.6 (5.5)	4.7 (1.5)	3.3 (0.8)	3.1 (0.7)	7.5 (2.5)
	$Sleep_{TOT}$	66.6 (8.7)	74.2 (13.7)	79.9 (6.4)	69.5 (8.0)	62.4 (11.1)
	SE	36.6 (4.8)	40.8 (8.7)	42.3 (3.9)	39.9 (4.3)	34.8 (6.1)

Values are means (SEM) based on 4 to 5 recording sessions. All values are time (T) in minutes, except rapid eye movement sleep periods and sleep efficiency which are expressed as the absolute number of rapid eye movement sleep periods (REMp) and the total sleep as a percentage of the time in the apparatus (SE). TIA refers to time in apparatus; W, wakefulness; REM_{LAT}, REM latency; $Sleep_{LAT}$, sleep latency; $Sleep_{TOT}$, total sleep time; SE is calculated as (100 X $Sleep_{TOT}$/TIA)

Table 2—Wakefulness and sleep in normoxia, hypocapnic, and isocapnic hypoxia across subjects

	Normoxia		Hypoxia		
Parameters	21% O_2	15% O_2	15% O_2+CO_2	10% O_2	10% O_2+CO_2
Time in wakefulness, %	49.7 (5.7)	42.1 (6.1)	43.2 (5.4)	55.6 (4.5)	47 (5.8)
Time in NREM sleep, %	34.9 (4.7)	41.1 (4.9)	41.4 (4.9)	41.1 (4.2)	42.1 (5.2)
Time in REM sleep, %	15.4 (1.2)	16.8 (1.8)	15.4 (1.1)	3.3 (1.0)*	10.9 (1.2)*†
REM sleep periods, no.	4.9 (0.5)	4.3 (0.5)	4.5 (0.4)	1.4 (0.4)*	3.6 (0.5)*†
REM sleep latency, min	32.4 (4.1)	22.4 (4.1)	28.1 (5.1)	107.5 (16.6)*	32.5 (5.2)†
Sleep latency, min	9.4 (2.3)	3.1 (0.5)	1.9 (0.6)*	4.6 (1.8)	4.1 (1.2)
Total sleep, min	90.9 (10.1)	102.6 (10.0)	102.0 (8.5)	80.2 (8.3)	97.7 (10.8)
Sleep efficiency, %	50.3 (5.7)	57.9 (6.1)	56.8 (5.4)	44.4 (4.5)	53 (5.8)

Values are reported as mean (SEM), based on 13 to 15 recording sessions (~180 min) in 3 cats.
*P < .05 compared to normoxia
†P < .05 compared to respective hypocapnic hypoxia

Data Analysis

Sleep and wakefulness were defined on the basis of standard EEG criteria. Epochs of 1.57 minutes were scored as wakefulness, NREM, or REM sleep on the basis of the predominant state during that epoch. The epoch length corresponded to 1 page of a recording on an AstroMed MT9500 recorder running at 3 mm per second. Although this is a long epoch compared to those used in human studies, it is adequate for discriminating sleep and wakefulness in the cat and necessary because of the large number of recordings required for the study. With this epoch length, more than 13,000 pages were scored.

The REM-sleep periods were the total number of periods with durations ≥ 1.57 minutes during the 3-hour recording session. The REM-sleep latency was the amount of time from the onset of recording to the first REM-sleep episode. Sleep latency was the amount of time from the onset of recording to either NREM or REM sleep. Total sleep ($Sleep_{TOT}$) was the total time in REM sleep plus the total time in NREM sleep. Time in the apparatus (TIA) in this study was the time the animals were recorded in a session. Sleep efficiency was calculated as (SleepTOT/TIA)·100. Data from sessions in which there were technical difficulties were discarded. This necessitated extension of the recording periods an additional week or more to obtain a particular treatment

on a particular day of the week. During these extra weeks, in addition to the lost days, "filler days" were randomly chosen and included to make a week complete. As a result, more than 1 day was sometimes recorded for a given condition on a specific day. In this case, the data from the additional days were averaged with the data from the other days under the same condition. Statistical comparisons of the number of trials (n=13-15 successful sessions in each condition) were made using repeated measures analysis of variance (ANOVA) and Bonferroni's correction was used when making multiple comparisons. Results were considered significant when *P* was less than .05.

We examined the occurrence of arousals and awakenings from NREM sleep in 1 cat on 1 day in each of the experimental conditions. In this analysis, we chose, arbitrarily, the sessions that occurred on Wednesdays. For example, of the 5 sessions in which the animal breathed 10% O_2, we chose for analysis the session that occurred on a Wednesday. Similarly, of the 5 control sessions, we analyzed the control session that occurred on a Wednesday. In this analysis, in each 1.57-minute epoch scored as NREM sleep, the number of arousal and awakenings were tabulated. Arousals were defined as desynchronizations of the EEG of at least 2 seconds and no more than 10 seconds. If the arousal was longer than 10 seconds, it was scored as an awakening. Awakenings and arousals in different conditions were analyzed with the Poisson distribution. In addition, they were correlated with the amount of REM sleep and the number of REM-sleep periods in that session. For analysis using the Poisson distribution, the expected number of arousals and awakenings under a given condition were calculated from the observed number of arousals and awakenings in the control (spontaneous breathing of room air) condition. This gave an expected value that could be compared with the actual value obtained under that condition.

Table 3—Wakefulness and sleep during spontaneous breathing and mechanical hyperventilation in individual subjects

Animal	Sleep Parameter	Spontaneous Breathing	Mechanical Ventilation 85% NREMθ	75% NREMθ	65% NREMθ
SUMR	TIA	171.9 (6.4)	179.5 (1.5)	177.3 (1.6)	176.9 (3.0)
	W_T	27.0 (6.0)	59.8 (6.4)	89.8 (10.8)	106.3 (23.0)
	$NREM_T$	111.5 (6.8)	99.9 (6.1)	79.9 (8.8)	64.7 (19.2)
	REM_T	33.4 (6.6)	19.8 (4.2)	7.5 (3.5)	6.0 (3.9)
	REM_P	6.4 (0.8)	4.2 (0.8)	1.9 (0.9)	2.1 (1.2)
	REM_{LAT}	22.1 (4.5)	22.0 (5.0)	84.6 (27.8)	94.0 (29.4)
	$Sleep_{LAT}$	5.8 (2.2)	8.8 (5.2)	5.7 (2.3)	14.4 (7.8)
	$Sleep_{TOT}$	144.9 (12.2)	119.6 (6.6)	87.5 (10.2)	70.7 (23.1)
	SE	83.8 (4.4)	66.7 (3.6)	49.4 (5.8)	39.9 (12.8)
CHDR	TIA	183.3 (8.2)	193.1 (10.3)	180.1 (0.8)	181.2 (3.0)
	W_T	91.5 (20.2)	98.5 (31.7)	93.6 (15.2)	135.0 (8.7)
	$NREM_T$	58.9 (18.7)	71.8 (26.5)	72.4 (13.7)	39.9 (5.0)
	REM_T	33.0 (7.1)	22.8 (7.9)	14.1 (2.6)	6.3 (2.1)
	REM_P	7.5 (1.2)	5.5 (1.4)	3.1 (0.5)	2.6 (0.8)
	REM_{LAT}	12.6 (2.9)	10.2 (4.0)	35.6 (17.8)	47.4 (37.8)
	$Sleep_{LAT}$	3.9 (3.4)	0.0 (0.0)	2.0 (2.1)	2.5 (2.4)
	$Sleep_{TOT}$	91.8 (15.5)	94.6 (30.9)	86.5 (14.5)	46.2 (5.7)
	SE	49.3 (12.5)	49.1 (16.7)	48.1 (8.2)	25.7 (3.7)
GWAY	TIA	175.9 (2.6)	178.6 (2.0)	169.8 (4.9)	177.8 (0.9)
	W_T	104.8 (15.5)	87.1 (10.2)	97.3 (5.3)	118.5 (3.7)
	$NREM_T$	46.3 (8.8)	68.7 (13.2)	58.1 (3.9)	48.7 (4.6)
	REM_T	24.7 (9.5)	22.8 (5.6)	14.3 (4.4)	10.6 (1.5)
	REM_P	3.4 (0.7)	2.5 (0.3)	2.8 (0.6)	2.5 (0.6)
	REM_{LAT}	55.3 (25.7)	48.3 (7.0)	46.1 (8.2)	69.9 (22.4)
	$Sleep_{LAT}$	7.1 (2.7)	1.2 (1.4)	2.4 (1.7)	2.4 (1.6)
	$Sleep_{TOT}$	71.0 (17.3)	91.5 (10.5)	72.4 (6.2)	59.3 (4.1)
	SE	40.2 (9.4)	51.2 (5.8)	42.6 (3.1)	33.3 (2.2)

Values are means (SEM) based on 4 to 5 recording sessions. All values are time (T) in minutes, except rapid eye movement sleep periods and sleep efficiency which are expressed as the absolute number of rapid eye movement sleep periods (REMp) and the total sleep as a percentage of the time in the apparatus (SE). NREMθ refers to non-rapid eye movement (NREM) eupneic end-tidal CO_2 level; TIA, time in apparatus; W, wakefulness; REM_{LAT}; rapid eye movement latency; $Sleep_{LAT}$, sleep latency; $Sleep_{TOT}$, total sleep time; SE is calculated as (100 X $Sleep_{TOT}$/TIA).

Table 4—Wakefulness and sleep during spontaneous breathing and mechanical hyperventilation across subjects

Parameters	Spontaneous breathing	Mechanical Hyperventilation 85% NREMθ	75% NREMθ	65% NREMθ
Time in wakefulness, %	40.2 (6.9)	43.5 (5.0)	53.0 (3.2)	67.1 (4.3)*
Time in NREM sleep, %	42.7 (5.7)	44.8 (4.7)	40.3 (3.0)	28.8 (3.8)
Time in REM sleep, %	17.1 (1.9)	11.7 (1.4)*	6.7 (1.0)*	4.2 (1.0)*
REM sleep periods, no.	5.8 (0.6)	4.1 (0.5)	2.6 (0.4)*	2.4 (0.5)*
REM sleep latency, min	29.4 (8.2)	26.5 (5.1)	56.1 (11.6)	70.5 (16.0)
Sleep latency, min	5.6 (1.3)	3.7 (2.1)	3.4 (1.1)	6.7 (2.9)
Total sleep, min	105.9 (12.5)	103.3 (8.9)	73.2 (5.8)*	58.7 (7.7)*
Sleep efficiency, %	59.8 (6.9)	56.5 (5.0)	47.0 (3.2)	32.9 (4.3)*

Values are means (SEM), based on 13-14 recording sessions in 3 cats. NREMθ refers to NREM eupneic end-tidal CO_2 level.
*P < .05 compared to spontaneous breathing.

RESULTS

Recording Time and Control Sleep

Results were obtained from 345 hours of recordings during which

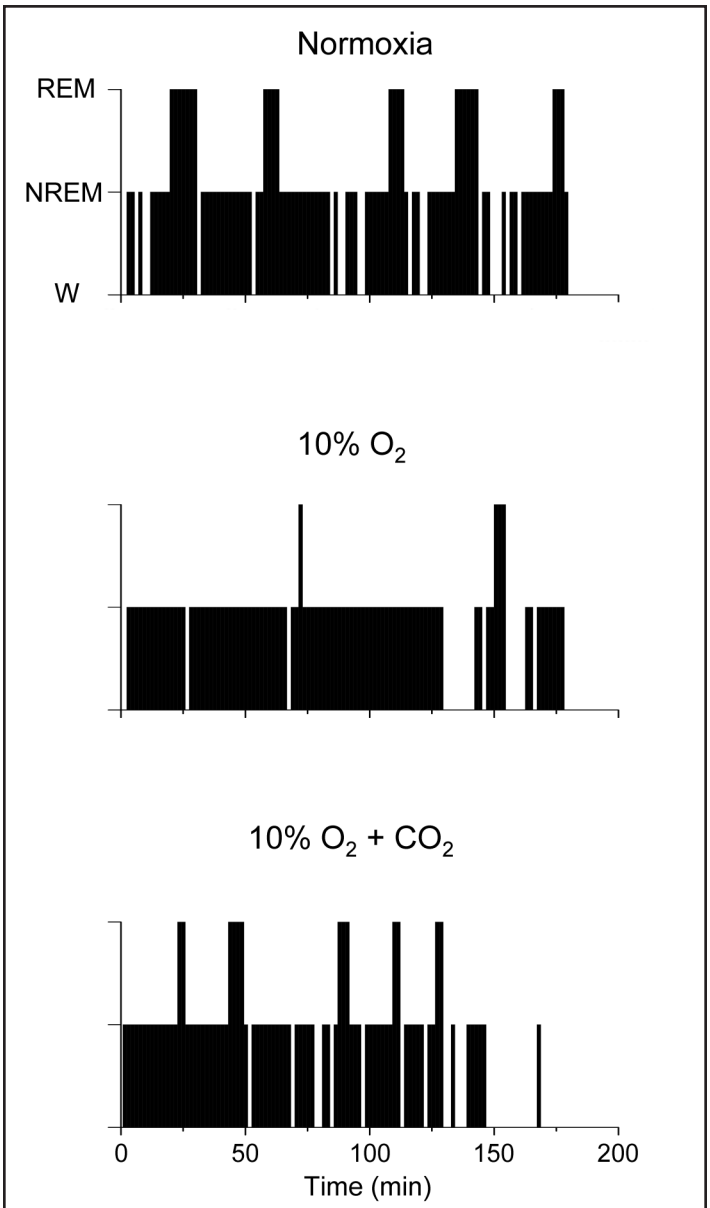

Figure 1—Sleep and wakefulness in a cat in normoxia, hypocapnic hypoxia (10% O_2), and isocapnic hypoxia (10% O_2 + CO_2). Level of hypoxia is 10% O_2 . Subject SUMR in Table 1. REM refers to rapid eye movement sleep; NREM, non-rapid eye movement sleep; W, wakefulness.

there were 475 REM-sleep periods. In all of the recordings, the animals slept swaddled in a veterinary cat bag with their head restrained. They breathed or were ventilated through a tube placed into the trachea. Under these conditions, the animals could not move freely and assume different postures, and the apparatus used to measure breathing or ventilate the animals may have caused some discomfort. The sessions were limited to approximately 3 hours. Under control conditions (spontaneously breathing room air, ie, 21% O_2) the animals slept 50% to 60% of the time in the apparatus. Rapid eye movement sleep occurred on average 15% to 17% of the time in the apparatus, which was 30% to 35% of the total sleep time. The means and the SEM of the measured parameters for sleep under control conditions are given in Tables 1 through 4. The control for the study using mechanical ventilation was also spontaneous breathing, as shown in Table 4. It was not possible to have a control in which the animals were ventilated with CO_2 at 100% of the normal level because this normal level of CO_2 elicited breathing against the ventilator. At CO_2 levels that were 85% of normal NREM sleep levels, sleep occurred on average 47% of the time, and REM sleep occurred approximately 12% of the time in the apparatus, which was 26% of the total sleep time.

Hypocapnic and Isocapnic Hypoxia

Compared to normoxia controls, hypocapnic hypoxia (10% O_2) significantly reduced REM sleep by approximately 80% and significantly increased REM-sleep latency (Figures 1 & 2, Tables 1 & 2). The reductions in REM sleep in hypocapnic hypoxia were caused by significant reductions in both the duration of the REM-sleep periods and the number of REM-sleep periods (Figures 1 & 2, Tables 1 & 2). When CO_2 was added to the inspirate to create isocapnic hypoxia, significant increases occurred in both time in REM sleep and the number of REM-sleep periods, and REM-sleep latency was significantly reduced (Figures 1 & 2, Tables 1 & 2). Nevertheless, the amount of REM sleep was still reduced by approximately 30% in isocapnic hypoxia compared to normoxia (Figures 1 & 2, Tables 1 & 2). The reductions in REM sleep were the result of significant reductions in both the duration of the REM-sleep periods and the number of REM-sleep periods (Figures 1 & 2, Tables 1 & 2).

The amount of wakefulness and NREM sleep, sleep latency, total sleep time, and sleep efficiency were not significantly affected by either hypocapnic hypoxia or isocapnic hypoxia (Figures 1 & 2, Tables 1 & 2).

Sleep disruption was not the result of disordered breathing. During hypoxia, there was a sustained hyperventilation throughout the 3-hour sessions with increases in both rate and depth of breathing. State-specific patterns of breathing were maintained such that, for example, breathing was rapid and irregular in REM sleep during hypoxia (Figure 3) just as it was in normoxia. Periodic breathing was not observed. Details on the pattern of breathing during hypoxia in sleep and wakefulness can be found in a separate report.[17]

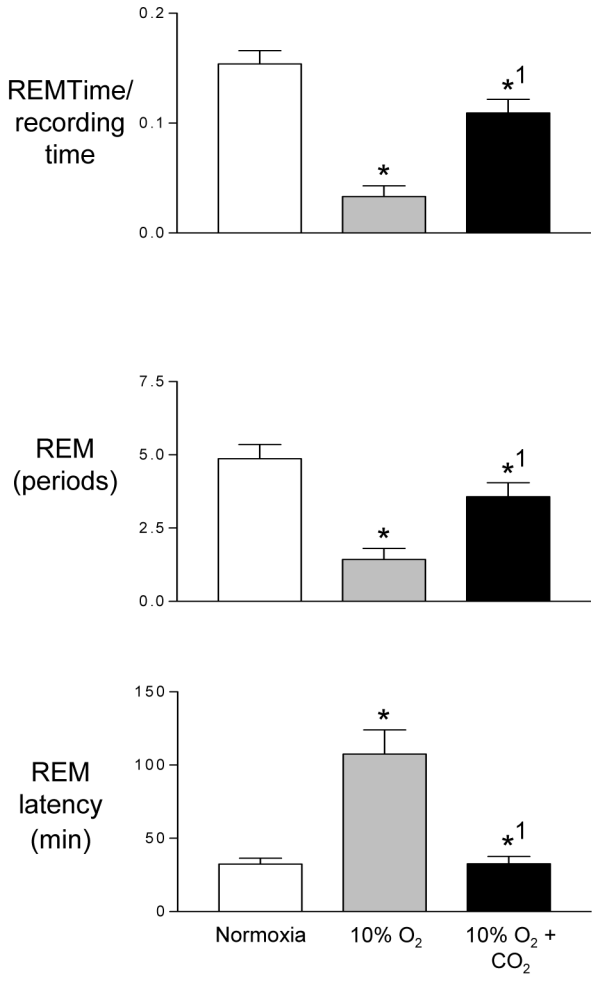

Figure 2—Rapid eye movement (REM) sleep in cats in normoxia, hypocapnic hypoxia (10% O_2), and isocapnic hypoxia (10% O_2 + CO_2). Values are mean ± SEM of the data from 3 cats. *P < .05 compared to room air, *1P < .05 compared to hypocapnic hypoxia.

Table 5—Awakenings and arousals during NREM sleep and duration of REM sleep and number of REM sleep periods in normoxia and hypoxia in one cat

	Normoxia	Hypoxia			
	21% O_2	15% O_2	15% O_2+CO_2	10% O_2	10% O_2+CO_2
Awakenings					
Actual events	5.2	21	14.8	14.7	26
Expected events	—	7	7.4	4.9	5.2
Cumulative Poisson probability	—	1.00*	0.99*	1.00*	1.00*
Arousals					
Actual events	62.4	70	148	53.9	166.4
Expected events	—	84	88.8	58.8	62.4
Cumulative Poisson probability	—	0.07	1.00*	0.25	1.00*
REM sleep					
Duration, min	47.1	37.7	33.0	18.8	11.0
Periods, no.	7	6	5	2	2

Data are from the Wednesday session under each condition in animal SUMR.
*P < .05 compared to normoxia using the Poisson distribution.
NREM refers to non-rapid eye movement; REM, rapid eye movement.

Table 6—Awakenings and arousals during NREM sleep and duration of REM sleep and number of REM sleep periods during spontaneous breathing and mechanical hyperventilation in one cat

	Spontaneous breathing	Mechanical Ventilation		
		85θ	75θ	65θ
Awakenings				
Actual events	24	31	18.5	11.4
Expected events	—-	18.6	11.1	5.7
Cumulative Poisson probability	—-	1.00*	0.98*	0.99*
Arousals				
Actual events	120	111.6	92.5	32.3
Expected events	—-	93	55.5	28.5
Cumulative Poisson probability	—-	0.97*	1.00*	0.78
REM sleep				
Duration, min	31.4	15.7	4.7	3.1
Periods, no.	7	4	1	2

Data are from the Wednesday session in each condition in animal SUMR. θ refers to the percentage of normal CO_2 level; NREM, non-rapid eye movement; REM, rapid eye movement.
*P < .05 compared to normoxia using the Poisson distribution.

Hypocapnia Induced by Mechanical Ventilation

Compared to REM sleep during spontaneous breathing, REM sleep during all levels of mechanically induced hypocapnia was significantly decreased (Figures 4 & 5, Tables 3 & 4). Rapid eye movement sleep occupied approximately 17% of the total recording time in spontaneously breathing animals. At hypocapnic levels that were 85% of the NREM-sleep levels, REM-sleep time was approximately 12% of the total recording time; at 75%, it was approximately 7% of the total recording time; and at 65%, it was approximately 4% of the total recording time. Thus REM-sleep disruption increased with increasing levels of hypocapnia.

The percentage of time spent in NREM sleep at any level of hypocapnia was not significantly different from the percentage of time in NREM sleep during spontaneous breathing. However, with extreme hypocapnia (65%), there was a significant increase in wakefulness and a trend toward a decrease in NREM sleep (Figures 4 & 5 & Tables 3 & 4). This trend was also evident in data showing significant reductions in total sleep time and sleep efficiency at 75% and 65% of the NREM-sleep levels.

Arousals and Awakenings

Tables 5 and 6 show the results of analyses of arousals and of awakenings of 1 cat (SUMR) on 1 of the days (Wednesday) in each of the 9 experimental conditions. Table 5 shows that there was not a significant increase in arousals in response to 15% and 10% O_2 compared to breathing of room air. However, there was a significant increase in arousals under conditions of 15% O_2 + CO_2 and 10% O_2 + CO_2. Furthermore, the number of awakenings was significantly greater under all hypoxic conditions. There were 11.5 awakenings per hour of NREM sleep in hypoxia compared with 4 awakenings per hour of NREM sleep in normoxia. The 10% O_2 + CO_2 data were outliers on this day in this cat. Overall, REM sleep was increased in this condition, but on this day and under these conditions, the animal was restless and did not sleep well.

Compared with spontaneous breathing, the number of arousals was increased during mechanical ventilation at 85% and 75% of the normal level of CO_2 but not at 65% (Table 6). The number of awakenings was significantly increased at all levels of CO_2 examined. During spontaneous breathing, there were 11.5 awakenings per hour of NREM sleep and 19, 19, and 23 awakenings per hour of NREM sleep at 85%, 75%, and 65% of the normal level of CO_2, respectively.

Across conditions, there was a strong negative correlation between the duration of REM sleep and the number of awakenings (correlation: -0.93). Similarly, there were negative correlations between the number of arousals and the duration of REM sleep in the session (-0.58), between the number of arousals and the number of REM-sleep periods (-0.55), and between the number of awakenings and the number of REM-sleep periods (-0.74).

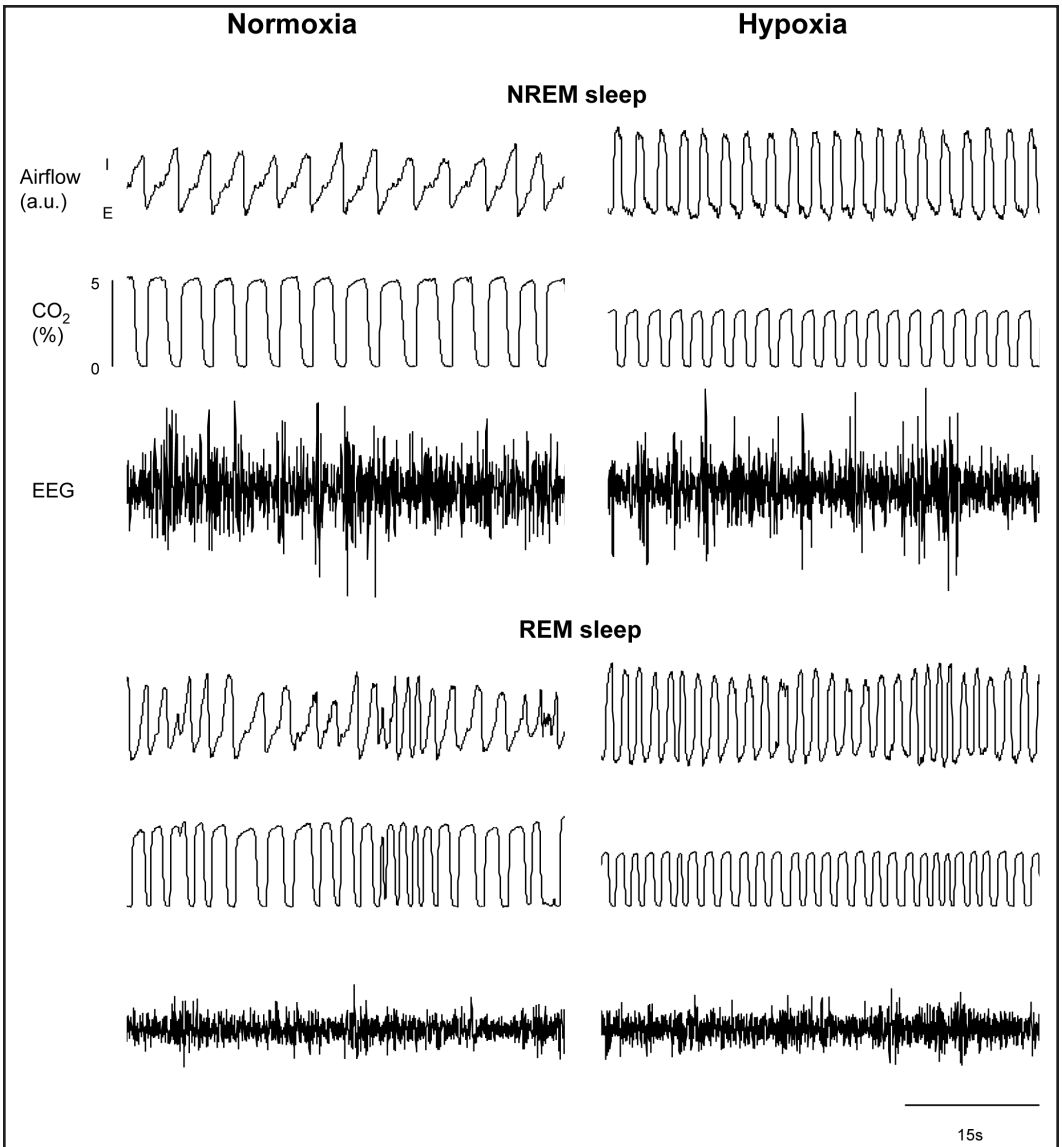

Figure 3—Breathing during non-rapid eye movement (NREM) and rapid eye movement (REM) sleep in normoxia and hypoxia. Traces are airflow, percentage of tidal CO_2, and electroencephalogram (EEG). I refers to inspiration; au, arbitrary units; E, expiration.

DISCUSSION

Hypocapnia disrupted REM sleep in both normoxic and hypoxic conditions. Decreased REM sleep occurred without disordered breathing but in association with an increased number of arousals and awakenings. Also, decreases in the amount of REM sleep occurred in response to hypocapnia induced by mechanical hyperventilation, and this, too, was associated with an increase in the number of awakenings and arousals. Wakefulness and NREM sleep were not affected by either hypocapnic hypoxia or isocapnic hypoxia, but wakefulness was increased and NREM sleep tended to decrease in hypocapnia induced by mechanical hyperventilation.

Sleep Disruption in Hypoxia

Hypoxic environments disrupt sleep.[1-3] Previous studies in the rat (a eutherian mammal) and the potoroo (a marsupial mammal) have reported that REM sleep decreased about 81% (range 76%-89%) in an environment of 10% O_2.[5,7,8,18] Our data showing a decrease of 80% at the same fraction of inspired O_2 confirm these earlier reports. The effect of hypoxia on NREM sleep is less clear. There are reports of decreases in REM sleep in rats (32%-47%),[5-7;18] but others report only slight increases.[8] We observed a slight increase in NREM sleep, but our values did not achieve statistical significance. Human studies have

shown that stages 1 and 2 NREM sleep are increased and stages 3 and 4 NREM sleep and REM sleep are decreased.[4,9,10;14]

Mechanism or Mechanisms of Hypoxia-induced Sleep Disruption

Periodic breathing is commonly seen in humans at high altitudes and may cause sleep disruption.[4,10,12-14] This pattern of breathing consists of waxing and waning breaths that can culminate in apnea and awakenings or arousals. However, in the present study, sleep was disrupted, but breathing was not periodic with apneas and associated awakenings. Similarly, in a marsupial, REM sleep is reduced in hypoxia in the absence of periodic breathing.[8]

Arousals and awakenings can occur in hypoxic environments independently of disordered breathing and may cause a reduction in the amount of REM sleep. Frequent (once per minute of sleep) brief arousals have been shown to reduce both REM sleep and psychomotor performance and increase sleepiness in normoxic adolescents.[19] In the current study, we examined the number of arousals and awakenings during NREM sleep in 1 cat in all 9 experimental conditions. We found that the number of REM-sleep periods and the duration of REM sleep in the experimental conditions were negatively correlated with the number of arousals and awakenings observed during the NREM-sleep periods. Anholm and associates[10,15] have suggested that brief arousals may be responsible for progressive worsening of subjective sleep quality as altitude increases. Our results support this idea and indicate, in addition, that hypocapnia rather than hypoxia may cause the brief arousals.

In the current study, we separated the effects of hypoxia and hypocapnia on sleep. Our results show that CO_2 improved sleep in hypoxic (10%

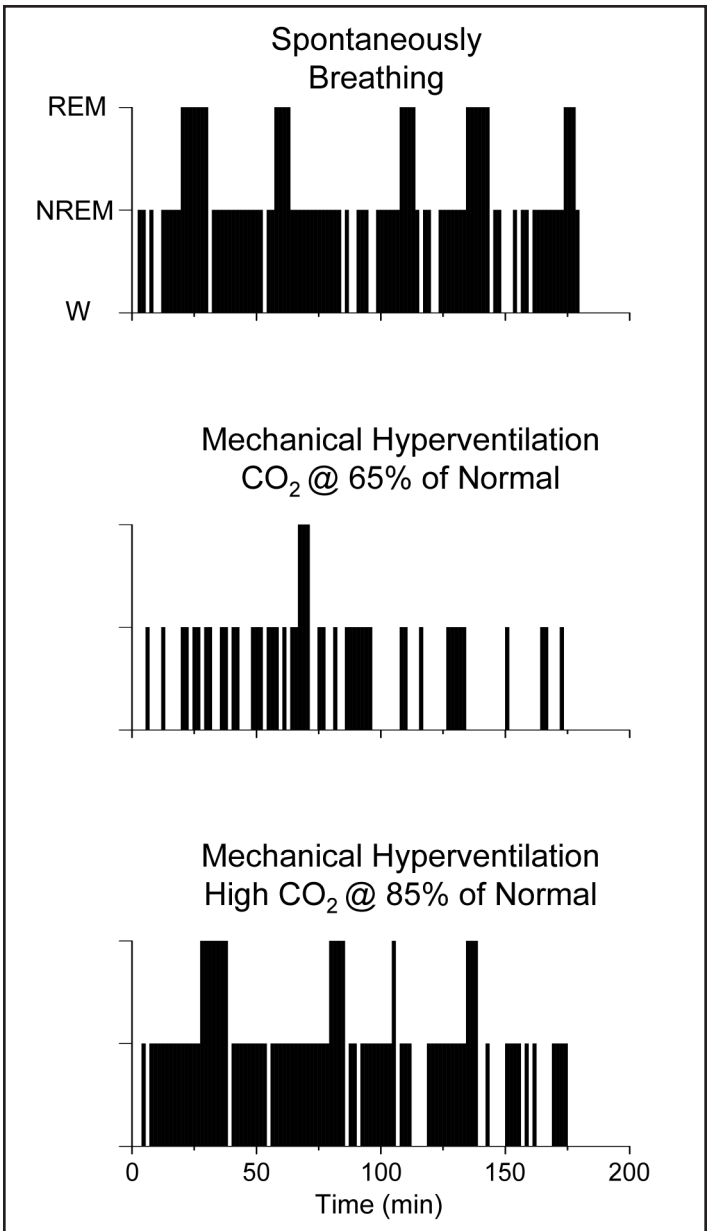

Figure 4—Sleep and wakefulness in a cat during spontaneous breathing and during mechanical hyperventilation with low CO_2 (65% of the non-rapid eye movement eupneic level) and high CO_2 (85% of the non-rapid eye movement eupneic level). Subject SUMR in Table 3. REM refers to rapid eye movement sleep; NREM, non-rapid eye movement sleep; W, wakefulness.

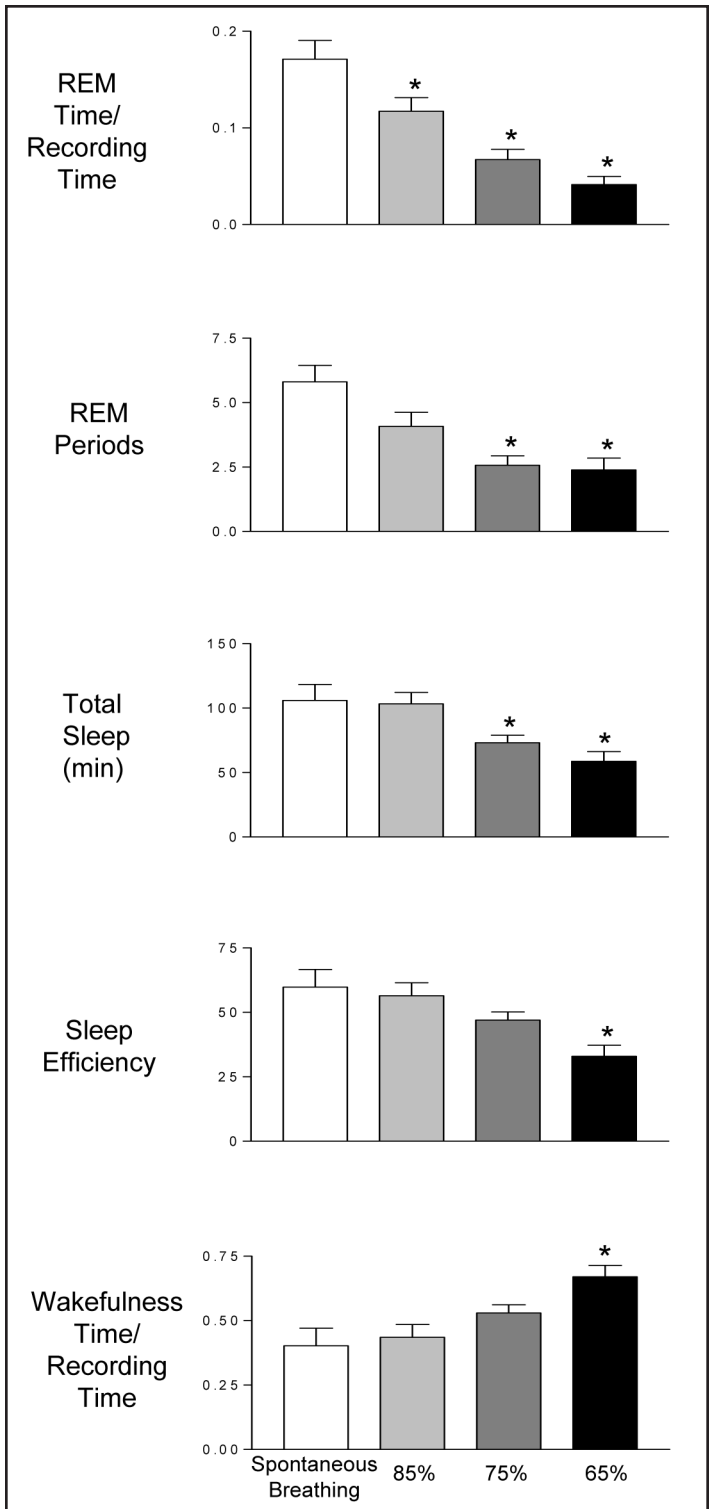

Figure 5—Sleep and wakefulness in cats during spontaneous breathing and during mechanical hyperventilation. During mechanical hyperventilation, end-tidal CO_2 was maintained at either 65%, 75%, or 85% of the non-rapid eye movement sleep eupneic level. Values are mean ± SEM of the data from 3 cats. *$P < .05$ compared to spontaneous-breathing room air. REM refers to rapid eye movement sleep.

O_2) conditions. Isocapnic hypoxic conditions restored total time spent in REM sleep to near-normal levels. Similarly, Megirian and colleagues[18,20] have shown that, in hypoxia, rats with denervated peripheral chemoreceptors have more REM sleep than do normal rats. Presumably, the denervated animals did not respond to the hypoxia and, therefore, were not hypocapnic. If so, these results support our findings of the importance of hypocapnia in the sleep disruption caused by hypoxia.

Other studies have found that the addition of CO_2 to the hypoxic gas mixtures does not improve sleep.[5-7] We cannot account for this difference in findings. The studies that claim that CO_2 does not improve sleep were performed on rats, whereas we studied cats. The rat, unlike the cat, responds to hypoxia by decreasing metabolism, which may affect the results. Nevertheless, our results with mechanical ventilation demonstrate conclusively that CO_2 affects sleep in cats. Hypocapnia decreased the amount of REM sleep at all levels examined, and the amount of decrease was proportional to the degree of the hypocapnia. We found further that hypocapnia significantly increased wakefulness and decreased NREM sleep, although not significantly. Ours is the first study to show that hypocapnia in normoxic conditions decreases the amount of REM sleep.

Carbon dioxide can have many physiologic effects that could, in turn, alter sleep. Cerebral blood flow varies inversely with the level of CO_2 and could possibly affect cerebral metabolism. Brainstem artery blood flow velocity decreases approximately 2.8% per mm Hg in man,[21] but, in cats, moderate hypocapnia (PCO_2 = 18.75 mm Hg) does not alter the regional cerebral metabolic rate of O_2 and does not cause tissue hypoxia.[22] In our cats, the average end-tidal CO_2 in NREM sleep during spontaneous breathing was 32.5 mm Hg (upper limit, 33.0 ± 0.1 mm Hg; lower limit 30.9 ± 0.1 mm Hg). Thus, 65% of the NREM eupneic level was 21.1 mm Hg, which is above the level at which hypocapnia causes tissue hypoxia, decreased regional cerebral metabolic rate of oxygen, or both. In unanesthetized cats, Neubauer and associates[23] have shown that blood flow to the medulla and pons is greater in hypoxia than in normoxia. Further, using levels of hypoxia analogous to those in our study, Krasney and associates[24] have shown that cerebral O_2 and glucose uptake in sheep is not affected by either hypocapnic or isocapnic hypoxia.

Low CO_2 levels cause an increased pH, which may affect sleep. Treatment with acetazolamide (a carbonic anhydrase inhibitor) improves sleep at high altitudes.[9,15] The efficacy of acetazolamide is attributed to stimulation of respiration (which increases PO_2) and prevention of sleep-disordered breathing.[15,25] However, inhibition of carbonic anyhdrase also results in a metabolic acidosis because of a bicarbonate diuresis.[26] Thus, although supplemental O_2, acclimatization, and the use of acetazolamide improve sleep, the mechanism responsible for the improvement is not apparent. It may involve normalization of pH.

CONCLUSION

Our results and others' have shown that hypoxia decreases the amount of REM sleep.[5-8,18,20] However, at high altitude, the body must cope with both hypoxia and hypocapnia. We show here that hypocapnia without hypoxia decreases the amount of REM sleep in cats. Thus, hypoxia-induced sleep disruption in cats is caused not only by low O_2 conditions, but also by low CO_2. This may also be the case with sleep disruption in humans at high altitude. There are known physiologic conditions in which O_2 is low, CO_2 is normal, and REM sleep occurs normally. In the last trimester, the human fetus spends a majority of its time in a REM-sleep-like state, and blood-gas analysis shows that O_2 levels are low but CO_2 levels are normal.[27] Similarly, the pouch of possums (*Didelphis virginiana* and *Trichosurus vulpecula*) has a gas content ranging from 14% to 16% O_2, with CO_2 concentrations ranging from 4% to 5%,[28,29] and these infant marsupials spend as much as 50% of their time in REM sleep.[30]

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