State-specific Effects of Sevoflurane Anesthesia on Sleep Homeostasis

Selective Recovery of Slow Wave but Not Rapid Eye Movement Sleep

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ABSTRACT

Background: Prolonged propofol administration does not result in signs of sleep deprivation, and propofol anesthesia appears to satisfy the homeostatic need for both rapid eye movement (REM) and non-REM (NREM) sleep. In the current study, the effects of sevoflurane on recovery from total sleep deprivation were investigated.

Methods: Ten male rats were instrumented for electrophysiologic recordings under three conditions: (1) 36-h *ad libitum* sleep; (2) 12-h sleep deprivation followed by 24-h *ad libitum* sleep; and (3) 12-h sleep deprivation, followed by 6-h sevoflurane exposure, followed by 18-h *ad libitum* sleep. The percentage of waking, NREM sleep, and REM sleep, as well as NREM sleep δ power, were calculated and compared for all three conditions.

Results: Total sleep deprivation resulted in significantly increased NREM and REM sleep for 12-h postdeprivation. Sevoflurane exposure after deprivation eliminated the homeostatic increase in NREM sleep and produced a significant decrease in the NREM sleep δ power during the postanesthetic period, indicating a complete recovery from the effects of deprivation. However, sevoflurane did not affect the time course of REM sleep recovery, which required 12 h after deprivation and anesthetic exposure.

Conclusion: Unlike propofol, sevoflurane anesthesia has differential effects on NREM and REM sleep homeostasis. These data confirm the previous hypothesis that inhalational agents do not satisfy the homeostatic need for REM sleep, and that the relationship between sleep and anesthesia is likely to be agent and state specific.

What We Already Know about This Topic

- Propofol appears to satisfy the need for both rapid eye movement (REM) and non-REM sleep

What This Article Tells Us That Is New

- After sleep deprivation in rats, 6 h sevoflurane exposure completely reverses non-REM sleep debt, but has no effect on REM sleep
- Inhalational anesthetics have different effects on sleep homeostasis and recovery than propofol

SLEEP and general anesthesia are distinct states that share a number of neurochemical and behavioral traits.[1–3] The relationship of sleep and anesthesia may reflect a shared genetic determinant, because *Drosophila* models with mutations of the *Shaker* voltage-gated potassium channel reveal that a single gene can control both sleep behavior and sensitivity to isoflurane.[4] Neurochemically, various anesthetic agents have been shown to act on neural systems involved in sleep–wake regulation.[2,3,5–13] Previous studies have also revealed a strong functional relationship between sleep and anesthesia.[7,14,15] Sleep deprivation has been demonstrated to reduce the time to anesthetic induction and delay the emergence from propofol and isoflurane anesthesia in rats.[15] Rats emerging from prolonged propofol administration do not show any signs of sleep deprivation,[14] whereas propofol administration in sleep-deprived rats ameliorates the effects of deprivation, such as increased sleep duration and intensity.[16] These findings indicate that propofol may modulate sleep homeostasis by satisfying the need for both nonrapid eye movement (NREM) and rapid eye movement (REM) sleep.

A recent study from our laboratory showed that isoflurane exposure in REM sleep-deprived rats is not associated with recovery of REM sleep.[17] This finding suggests that inhala-

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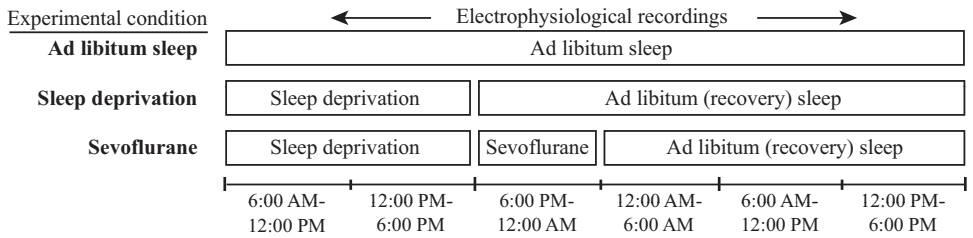

Fig. 1. Schematic showing the experimental design and the temporal order (from top to bottom) of experimental interventions for sleep–wake recordings under (1) *ad libitum* sleep, (2) 12 h of sleep deprivation, and (3) 12 h of sleep deprivation followed by 6 h of sevoflurane exposure.

tional agents may have an interface with sleep that is distinct from intravenous drugs, such as propofol, which is consistent with recent studies demonstrating that anesthetic agents differ in their interaction with structures that regulate sleep–wake behavior.[7,18] In the current study, we tested the hypotheses that (1) sevoflurane anesthesia would have a differential effect on the homeostatic recovery of NREM and REM sleep from sleep deprivation and (2) sleep deprivation would reduce the time to anesthetic induction.

Materials and Methods

The experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals (Ann Arbor, Michigan) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All the experiments were conducted on male (n = 10) Sprague-Dawley rats (mean weight = 338.5 g) (Harlan, Indianapolis, IN). The rats were maintained on a 12-h light:12-h dark cycle (lights on at 6:00 AM) and were provided *ad libitum* food and water. Under surgical anesthesia (ketamine 65 mg/kg, xylazine 10 mg/kg, intraperitoneal delivery), the rats were implanted with electrodes for sleep–wake electrophysiologic recordings. The electroencephalogram was recorded through the screw electrodes implanted bilaterally in the frontal (AP: +0.3 mm; ML: ±2.0 mm, bregma) and parietal areas (AP: −3.0 mm; ML: ±2.0 mm, bregma). The electromyogram was recorded through flexible, insulated (except at the tip) multistranded wires (Cooner Wires, Chatsworth, CA) inserted into the dorsal nuchal muscle. The electrodes were interfaced with a six-pin pedestal (Plastics One, Roanoke, VA), and the entire assembly was secured on the skull using dental acrylic.

Experimental Interventions

After a minimum of 7 days of postsurgical recovery period, the rats were habituated to the recording setup on 3 separate days for 3 h each day. During the habituation, the electroencephalogram and electromyogram were recorded from freely moving rats to ensure that the signals were artifact free. Electrophysiologic signals from one rat were found to be unusable because of the movement artifacts. Data from this rat were not included in sleep–wake state analyses. After habituation to the recording setup, the same rats (n = 9) underwent three different experimental interventions (in the order depicted in fig. 1).

1. Ad Libitum Sleep–Wake Group. Electroencephalogram and electromyogram were continuously recorded from freely moving rats for 36 h under normal conditions.

2. Sleep-Deprivation Group. Starting at the onset of the light cycle (6:00 AM), the rats were subjected to 12 h of total sleep deprivation through gentle handling. During the sleep-deprivation procedure, the rats were allowed to move around freely while the electroencephalogram and electromyogram were being recorded. The rats were gently manipulated if they assumed a sleeping posture or if the electroencephalogram showed the appearance of high-amplitude, slow-frequency waves. After 12 h of sleep deprivation, the rats were allowed *ad libitum* recovery sleep and the electrophysiologic recordings were continued for another 24 h.

3. Sevoflurane Group. The rats underwent 12 h of sleep deprivation (gentle handling) during the light phase (6:00 AM–6:00 PM). The electrophysiologic signals were recorded during sleep deprivation and for the rest of the experimental procedure. Immediately after the sleep deprivation, the rats were anesthetized with 3.0% sevoflurane in 100% oxygen. Induction was carried out in a clear acrylic rectangular chamber that was equilibrated with 3.0% sevoflurane for 3 min before introducing the rat in the chamber. The time to loss-of-righting reflex (LORR) was recorded after the animal was placed in the chamber. Thereafter, the rat was connected to a nonrebreathing gas circuit, and a steady flow of sevoflurane in 100% oxygen was delivered *via* a nose cone. Sevoflurane concentrations were titrated to achieve a burst suppression ratio of approximately 50%. This anesthetic-specific endpoint of burst suppression was chosen to ensure that all animals were exposed to a similar level of anesthetic depth and to preclude the possibility of animals entering the state of NREM sleep. Animals were observed continuously during general anesthesia, and the inspired gas concentrations were monitored using an inline Datex Capnomac Ultima™ gas analyzer (Datex Medical Instrumentation, Tewksbury, MA). Mean sevoflurane concentration required to achieve 50% burst suppression was 3.38 ± 0.03% (range: 2.9–3.8). The heart rate, respiratory rate, and oxygen saturation were continuously monitored using a MouseOx® monitor (Starr Life

Sciences, Oakmont, PA). Mean heart rate was 347.0 ± 1.0 beats/min (range: 331–360), and mean respiratory rate was 63.0 ± 0.77 breaths/min (range: 52–79). Mean oxygen saturation was 98.4 ± 0.03% (range: 97.2–98.7). Rectal temperature was monitored continuously, and the core body temperature was maintained at 37.6 ± 0.1°C (range: 37.0–38.4) through a water-based heating system (TP-500 Heat Therapy Pump; Gaymar Industries, Orchard Park, NY). In general, the animals tolerated the anesthetic exposure well. Because general anesthesia was administered during the first 6 h of the dark cycle (6:00 PM–12:00 AM), the anesthetic administration and the associated procedures were done under a red light source. After sevoflurane exposure was completed, animals were returned to their home chambers and recordings were continued for another 18 h.

In order to rule out the possibility of one intervention influencing the results of subsequent interventions, each intervention was followed by a recovery period of 4–6 weeks. During the recovery period, the rats stayed in their home cages under the same light-dark cycle. Further, to avoid any unknown bias due to the order of the experimental interventions, sevoflurane treatment—the only pharmacologic intervention—was always performed last. In addition, after the completion of the sleep-deprivation study, the time to LORR was also measured in the rats under non–sleep-deprived conditions, which was statistically compared with the time to LORR in rats undergoing sleep deprivation. The experimental procedure, sevoflurane induction concentration (3.0%), and the time of measurements (6:00 PM) were the same as those for the sleep-deprived rats.

Electrophysiologic Recording and Sleep–Wake Analyses

Electrophysiologic signals were recorded using Lynx-8 amplifiers interfaced with Cheetah Data Acquisition™ software (Neuralynx, Bozeman, MT). The signals were filtered between 0.1 and 125 Hz and sampled at 667 Hz. The screw electrode on the left frontal bone was used as a reference electrode for bipolar differential electroencephalographic recordings from the right frontal and left parietal hemisphere. The electroencephalogram from the frontal-frontal and frontal-parietal pairs of electrodes, as well as nuchal muscle electromyogram, was used to characterize different sleep–wake states. The frontal-parietal electroencephalographic signals were used for calculating δ power using the Matlab Signal Processing Toolbox™ (The MathWorks, Natick, MA). The spectral density for NREM sleep epochs was estimated using the Welch method (implemented in the Matlab Signal Processing Toolbox). The Welch method reduces the influence of noise and finite size effect, compared with other methods. We used a hamming window and calculated results with 21 windows, each 2 s long, that overlapped consecutively by 80%. In order to minimize the within-group variability, the power spectral data were normalized by dividing the δ power (0.5–4 Hz) for each NREM sleep epoch with the epoch's power in the 0.4- to 20-Hz range.

For sleep staging, the electroencephalogram was bandpass filtered between 0.1 and 30 Hz, whereas the electromyogram was bandpass filtered between 30 and 125 Hz. Sleep–wake states were scored in 10-s bins and categorized as waking, NREM sleep, or REM sleep. Sleep scoring was performed using an automated Matlab-based sleep-scoring software,[19] which was subjected to random manual checks by three blinded experimenters. The waking state was identified by the presence of (1) low-voltage, high-frequency electroencephalogram, (2) prominent θ activity (4–9 Hz), and (3) active movements and high muscle tone. NREM sleep was characterized by the presence of high-voltage, low-frequency electroencephalographic signals, along with a reduced muscle tone. REM sleep was marked by (1) low-voltage, high-frequency electroencephalogram (frontal electrodes), (2) prominent θ activity (4–9 Hz, parietal electrodes), and (3) muscle atonia.

Statistical Analyses

The statistical analyses were performed using the software package, GraphPad Prism 5.01™ (La Jolla, CA). The data were confirmed to be normally distributed (D'Agostino and Pearson test). Percentages of sleep–wake states were calculated in blocks of 6 h for the entire recording period in all three conditions (*i.e.*, *ad libitum* sleep, sleep deprivation, and sevoflurane). A two-tail paired *t* test was used (1) to compare the time to LORR upon induction with sevoflurane in the sleep-deprived and *ad libitum*/nonsleep-deprived conditions and (2) to compare sleep–wake states as well as normalized δ power between the sleep-deprived and *ad libitum* sleep groups during the first 6 h of post–sleep-deprivation period. For the rest of the recording period, sleep–wake states as well as normalized δ power in temporally comparable blocks were statistically compared across groups using repeated measures analysis of variance (RMANOVA), and the *post hoc* pairwise multiple comparisons were performed with Bonferroni *post hoc* test. All the data are reported as mean ± SEM along with the 95% confidence interval (CI). A P value less than 0.05 was considered statistically significant.

Results

Validation of Sleep-Deprivation Protocol

A RMANOVA showed that total sleep deprivation through gentle handling had a significant effect on the time spent in the waking state ($F[2,24] = 88.35$, $P < 0.001$), NREM sleep ($F[2,24] = 64.09$, $P < 0.001$), and REM sleep ($F[2,24] = 39.23$, $P < 0.001$) during the first 6 h of sleep deprivation (6:00 AM–12:00 PM). A similar change in the time spent in the waking state ($F[2,24] = 59.81$, $P < 0.001$), NREM sleep ($F[2,24] = 32.51$, $P < 0.001$), and REM sleep ($F[2,24] = 75.61$, $P < 0.001$) was observed during the second 6 h of sleep deprivation (12:00 PM–6:00 PM).

Table 1. Percentage of Sleep–Wake States across *Ad Libitum* Sleep, Sleep Deprivation, and Sevoflurane Conditions during 12 h of Sleep Deprivation and the Immediate 6 h of Sleep Recovery Period

	6:00 AM–12:00 PM			12:00 PM–6:00 PM			6:00 PM–12:00 AM			
	Sleep Deprivation			Sleep Deprivation			Recovery			Under anesthesia
	Adlib Sleep	Sleep Dep	Sevoflurane	*Adlib* Sleep	Sleep Dep	Sevoflurane	*Adlib* Sleep	Sleep Dep	Sevoflurane	
Wake, %	54.0 ± 2.7 (47.8–60.2)	93.6 ± 2.2*** (88.5–98.5)	93.5 ± 2.0*** (88.8–98.1)	41.6 ± 3.0 (34.6–48.5)	86.0 ± 3.7*** (77.4–94.4)	86.8 ± 3.2*** (79.3–94.1)	83.8 ± 1.9 (79.4–88.2)	48.0 ± 5.6*** (35.07–60.9)		
NREM sleep, %	40.6 ± 3.0 (33.7–47.4)	6.4 ± 2.2*** (1.4–11.4)	6.4 ± 2.0*** (1.8–10.8)	44.2 ± 2.8 (37.7–50.6)	13.9 ± 3.7*** (5.4–22.3)	13.1 ± 3.2*** (5.7–20.3)	12.2 ± 1.5 (8.7–15.5)	36.1 ± 4.5*** (25.8–46.3)		
REM sleep, %	5.4 ± 0.8 (3.4–7.2)	0.02 ± 0.0*** (−0.01–0.05)	0.1 ± 0.1*** (−0.08–0.32)	14.2 ± 1.6 (10.4–17.9)	0.1 ± 0.1*** (−0.03–0.2)	0.2 ± 0.1*** (−0.008–0.31)	4.0 ± 0.7 (2.4–5.6)	15.9 ± 1.4*** (12.6–19.1)		

Data are reported as mean ± SEM. The values in parentheses are the 95% CI. Significant, compared with *adlib* sleep: *** $P < 0.001$. *Adlib* = *ad libitum*; Dep = deprivation; NREM = nonrapid eye movement; REM = rapid eye movement.

The time spent in waking showed a significant increase from 54.0 ± 2.7% (95% CI: 47.8–60.2) to 93.6 ± 2.2% (95% CI: 88.5–98.5) (Bonferroni *post hoc*, $P < 0.001$) in the first 6 h of deprivation (6:00 AM–12:00 PM) and from 41.6 ± 3.0% (95% CI: 34.6–48.5) to 86.0 ± 3.7% (95% CI: 77.4–94.4) (Bonferroni *post hoc*, $P < 0.001$) in the second 6 h of deprivation (12:00 PM–6:00 PM) (table 1). The increase in the waking state was associated with a significant decrease in NREM sleep from 40.6 ± 3.0% (95% CI: 33.7–47.4) to 6.4 ± 2.2% (95% CI: 1.4–11.4) (Bonferroni *post hoc*, $P < 0.001$) in the first 6 h of deprivation and from 44.2 ± 2.8% (95% CI: 37.7–50.6) to 13.9 ± 3.7% (95% CI: 5.4–22.3) (Bonferroni *post hoc*, $P < 0.001$) in the second 6 h of deprivation (table 1). There was a near total elimination of REM sleep, which decreased from 5.4 ± 0.8% (95% CI: 3.4–7.2) to 0.02 ± 0.0% (95% CI: −0.01–0.05) (Bonferroni *post hoc*, $P < 0.001$) in the first 6 h of deprivation and from 14.2 ± 1.6% (95% CI: 10.4–17.9) to 0.1 ± 0.1% (95% CI: −0.03–0.2) (Bonferroni *post hoc*, $P < 0.001$) in the second 6 h of deprivation (table 1). With the temporal progression of the light phase and sleep deprivation, the animals became more difficult to arouse and, consequently, spent more time in NREM sleep during the second 6-h block, compared with the first 6-h block.

A significant increase in NREM sleep and REM sleep was observed during the first 12 h of post–sleep-deprivation period (tables 1 and 2). During the first 6 h of post–sleep-deprivation period, the time spent awake was significantly reduced from 83.8 ± 1.9% (95% CI: 79.4–88.2) to 48.0 ± 5.6% (95% CI: 35.07–60.9) (t[8] = 6.29, $P < 0.001$), whereas the time spent in NREM sleep increased from 12.2 ± 1.5% (95% CI: 8.7–15.5) to 36.1 ± 4.5% (95% CI: 25.8–46.3) (t[8] = 5.61, $P < 0.001$), and the time spent in REM sleep increased from 4.0 ± 0.7% (95% CI: 2.4–5.6) to 15.9 ± 1.4% (95% CI: 12.6–19.1) (t[8] = 7.51, $P < 0.001$) (table 1). Significant increases in NREM sleep (F[2,24] = 15.46, $P < 0.001$) and REM sleep (F[2,24] = 29.19, $P < 0.001$) were also observed during the second 6-h block of the dark cycle (12:00 AM–6:00 AM), during which the recovery from sleep deprivation was completed (table 2). However, the maximal sleep rebound occurred in the first 6 h after total sleep deprivation (6:00 PM–12:00 AM), which therefore was chosen for sevoflurane exposure.

Effect of Sleep Deprivation on Sevoflurane Induction Time

The sevoflurane induction time, as measured by the LORR, was significantly reduced from 212.6 ± 9.4 s (95% CI: 191.3–233.9) in the non–sleep-deprived/*ad libitum* sleep group to 176 ± 14.3 s (95% CI: 143.7–208.3) in the sleep-deprived group (t[8] = 2.439, $P = 0.037$) (fig. 2).

Table 2. Percentage of Sleep–Wake States across *Ad Libitum* Sleep, Sleep Deprivation, and Sevoflurane Conditions during Post–Sleep Deprivation and Postanesthetic Recovery Period

	12:00 AM–6:00 AM			6:00 AM–12:00 PM			12:00 PM–6:00 PM		
	Adlib Sleep	Sleep Dep	Sevoflurane	*Adlib* Sleep	Sleep Dep	Sevoflurane	*Adlib* Sleep	Sleep Dep	Sevoflurane
Wake, %	74.4 ± 3.4 (66.4–82.2)	42.4 ± 4.4*** (32.2–52.5)	62.5 ± 3.8## (53.8–71.2)	32.4 ± 2.7 (26.1–38.6)	26.5 ± 2.4 (20.8–32.1)	20.7 ± 1.9** (16.3–25.1)	33.5 ± 2.8 (26.9–40.0)	43.8 ± 4.8 (32.6–54.8)	31.3 ± 2.1# (26.4–36.2)
NREM sleep, %	20.1 ± 2.6 (14.2–26.0)	40.2 ± 3.2*** (32.7–47.5)	25.9 ± 3.5## (17.8–33.8)	55.8 ± 2.5 (50.0–61.6)	60.0 ± 2.2 (54.8–65.1)	59.8 ± 1.3 (56.9–62.7)	46.8 ± 2.2 (41.8–51.7)	43.4 ± 3.6 (35.0–51.7)	51.2 ± 1.7 (47.3–54.9)
REM sleep, %	5.6 ± 1.2 (2.8–8.2)	17.4 ± 1.8*** (13.2–21.6)	11.6 ± 1.5**## (8.1–15.1)	11.8 ± 1.5 (10.9–12.6)	13.5 ± 1.1 (10.9–16.0)	19.4 ± 1.8**# (15.1–23.7)	19.8 ± 1.9 (15.4–24.0)	12.8 ± 1.8* (8.6–16.9)	17.5 ± 2.0 (13.0–22.0)

Data are reported as mean ± SEM. The values in parentheses are the 95% CI. Significant, compared with *ad libitum* sleep (*Adlib* sleep): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significant, compared with sleep deprivation (Sleep Dep): # $P < 0.05$, ## $P < 0.01$.

NREM = nonrapid eye movement; REM = rapid eye movement.

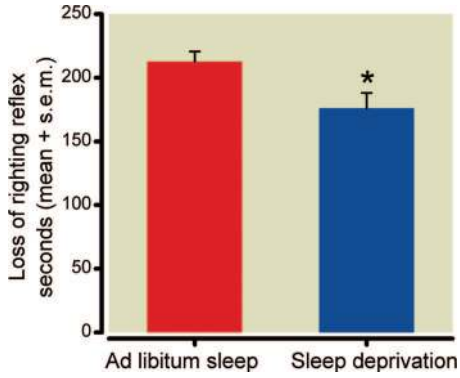

Fig. 2. Effect of sleep deprivation on the sevoflurane induction time as measured by time to loss of righting reflex. * $P < 0.05$ significant, as compared with *ad libitum* sleep.

Effect of Sevoflurane Anesthesia on Recovery from Sleep Deprivation

Comparison (RMANOVA) of the percentage of NREM sleep across the three conditions (*i.e.*, *ad libitum*, sleep deprivation, and sevoflurane) during the first 6 h of postanesthetic recovery period showed a significant difference between the three groups ($F[2,24] = 15.46$, $P < 0.001$). The time spent in NREM sleep after sevoflurane exposure was not significantly different compared with the *ad libitum* sleep group, in which no deprivation occurred (fig. 3). However, the time spent in NREM sleep in the sevoflurane group was significantly reduced (Bonferroni *post hoc*, $P < 0.01$) compared with the rats recovering naturally from sleep deprivation (fig. 3). There was no significant difference in the percentage of NREM sleep during the remaining 12-h period across the three groups ([$F(2,24) = 1.69$, $P = 0.21$] for 6:00 AM–12:00 PM and [$F(2,24) = 2.92$, $P = 0.08$] for 12:00 PM–6:00 PM) (fig. 3). Thus, sevoflurane exposure to sleep-deprived rats was associated with an accelerated recovery, accomplishing complete NREM sleep restoration during the 6 h of anesthesia, compared with 12 h of *ad libitum* sleep–wake states.

Analysis of the percentage of time spent in REM sleep (RMANOVA) during the 12 postanesthesia hours showed a

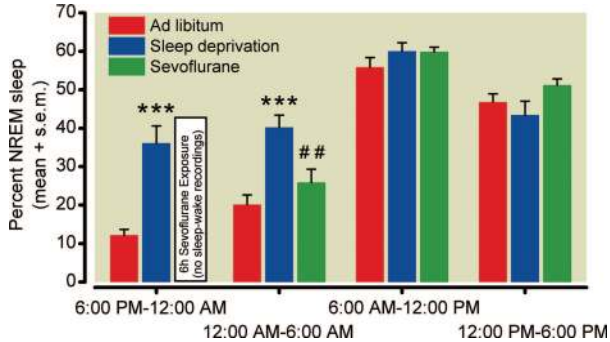

Fig. 3. Effect of sevoflurane exposure on the recovery of NREM sleep after total sleep deprivation. Note that sevoflurane exposure completely eliminates signs of NREM sleep rebound. *** $P < 0.001$ significant, compared with *ad libitum* sleep; ## $P < 0.01$ significant, compared with sleep deprivation alone. NREM = nonrapid eye movement.

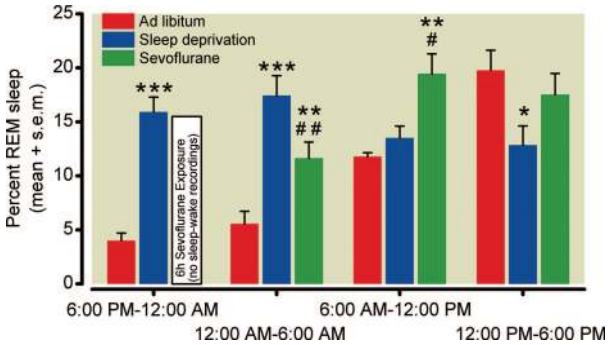

Fig. 4. Effect of sevoflurane exposure on the recovery of REM sleep after total sleep deprivation. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ significant, compared with *ad libitum* sleep; ## $P < 0.01$, # $P < 0.05$ significant, compared with sleep deprivation alone. REM = rapid eye movement.

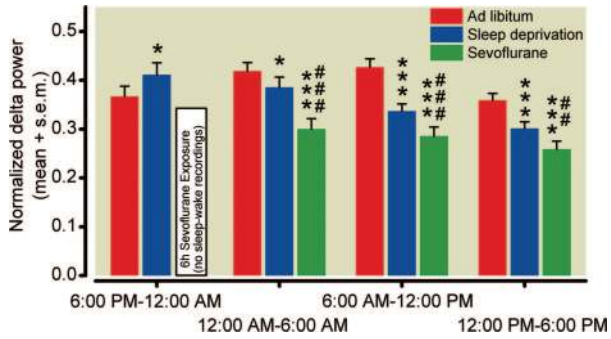

Fig. 5. Normalized δ power during post–sleep-deprivation and postanesthetic recovery period. *** $P < 0.001$, * $P < 0.05$ significant, compared with *ad libitum* sleep; ### $P < 0.001$, ## $P < 0.01$ significant, compared with sleep deprivation alone.

significant difference between *ad libitum*, sleep deprivation, and sevoflurane conditions ([$F(2,24) = 29.19$, $P < 0.001$] for 12:00 AM–6:00 AM and [$F(2,24) = 8.75$, $P = 0.002$] for 6:00 AM–12:00 PM) (fig. 4). The time spent in REM sleep increased from 5.6 ± 1.2% (95% CI: 2.8–8.2) to 11.6 ± 1.5% (95% CI: 8.1–15.1) (Bonferroni *post hoc*, $P < 0.01$) during the first 6 h postanesthesia and increased from 11.8 ± 0.4% (95% CI: 10.9–12.6) to 19.4 ± 1.8% (95% CI: 15.1–23.7) (Bonferroni *post hoc*, $P < 0.01$) in the subsequent 6-h block (fig. 4). Therefore, compared with NREM sleep, the effects of sevoflurane on recovery from REM sleep had a distinct profile. REM sleep recovery commenced only after the cessation of sevoflurane exposure, a finding consistent with our previous study of isoflurane.[17]

Effect of Sevoflurane Anesthesia on δ Power during NREM Sleep

Compared with the *ad libitum* sleep group (0.36 ± 0.02, 95% CI: 0.31–0.41), the sleep-deprived rats (0.41 ± 0.02, 95% CI: 0.35–0.46) showed a statistically significant increase in δ power ($t[8] = 2.764$, $P = 0.002$) during the immediate 6 h of post–sleep-deprivation recovery period (6:00 PM–12:00 AM) (fig. 5). This is consistent with the occurrence of rebound NREM sleep during the same time block (fig. 3). During the immediate 6 h of the postanesthesia period (12:00 AM–6:00 AM), there was a significant decrease in the δ power ($F[2,24] = 66.83$, $P < 0.001$). The rats exposed to 6 h of sevoflurane (0.30 ± 0.02, 95% CI: 0.25–0.34) following 12 h of sleep deprivation showed a statistically significant decrease in δ power, compared with the *ad libitum* sleep group (0.41 ± 0.01, 95% CI: 0.37–0.45) (Bonferroni *post hoc*, $P < 0.001$) as well as the sleep-deprivation group (0.38 ± 0.02, 95% CI: 0.33–0.43) (Bonferroni *post hoc*, $P < 0.001$).

The δ power during the subsequent 12 h for both the sleep-deprivation and sevoflurane groups remained significantly lower, compared with the *ad libitum group*. The δ power decreased for the sleep-deprivation group ([0.33 ± 0.01, 95% CI: 0.30–0.37] for 6:00 AM–12:00 PM and [0.30 ± 0.01, 95% CI: 0.26–0.33] for 12:00 PM–6:00 PM) and sevoflurane group ([0.28 ± 0.01, 95% CI: 0.24–0.32] for 6:00 AM–12:00 PM and [0.25 ± 0.01, 95% CI: 0.22–0.29] for 12:00 PM–6:00 PM), compared with the *ad libitum group* ([0.42 ± 0.01, 95% CI: 0.38–0.46] for 6:00 AM–12:00 PM and [0.035 ± 0.01, 95% CI: 0.32–0.39] for 12:00 PM–6:00 PM) ([$F(2,24) = 88.99$, $P < 0.001$, Bonferroni *post hoc*, $P < 0.001$] for 6:00 AM–12:00 PM and [$F(2,24) = 50.15$, $P < 0.001$, Bonferroni *post hoc*, $P < 0.001$] for 12:00 PM–6:00 PM) (fig. 5).

Discussion

The current study has three main findings. First, sevoflurane exposure titrated to an electroencephalographic endpoint of deep anesthesia is associated with a profound effect on NREM sleep homeostasis, eliminating signs of NREM sleep deprivation in half the time as *ad libitum* sleep. Second, sevoflurane anesthesia does not satisfy the homeostatic need for REM sleep, confirming our previous findings with isoflurane that REM sleep recovery begins after the completion of inhalational anesthetic exposure.[17] These data suggest that inhalational agents have differential effects on sleep homeostasis, which are further distinct from previous studies showing both NREM and REM sleep recovery during propofol exposure.[16] Finally, like both propofol[15] and isoflurane,[15] the effects of sevoflurane are potentiated by sleep deprivation.

Tung *et al.*[16] discussed three general categories for the relationship of sleep homeostasis and anesthesia. First, anesthesia could satisfy the homeostatic need for sleep. Second, anesthesia could, like the waking state, be associated with the accrual of sleep debt. Third, anesthesia could be a state unlike sleep or waking, resulting in a neutral effect on sleep debt. Tung *et al.*[16] showed that propofol best fits the first category, because it satisfied both NREM and REM sleep debt to a similar level as observed with *ad libitum* sleep. The current findings suggest that sevoflurane has state-specific effects on NREM and REM sleep homeostasis and thus does not fit into a single category (fig. 6).

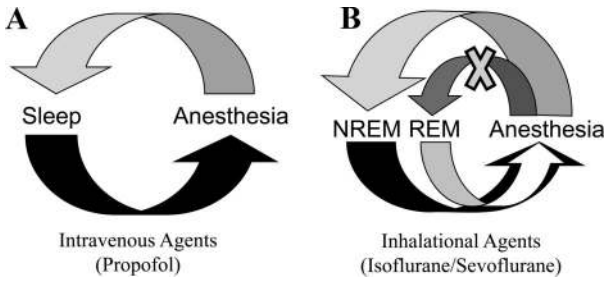

Fig. 6. Based on the available data, we propose the following models for interactions of general anesthesia and sleep homeostasis. (*A*) Propofol satisfies both NREM and REM sleep debt as effectively as natural sleep,[14,16] and sleep deprivation potentiates the effects of propofol,[15] which suggests a balanced, reciprocal relationship between sleep and anesthesia. By contrast, inhalational agents (*B*) have a differential effect on NREM and REM sleep. Based on the observed effects of sevoflurane, the influence on NREM sleep homeostasis appears to be profound, denoted here by the larger arrow. However, REM sleep recovery occurs only after the cessation of anesthetic exposure (lack of effect denoted by the "X"), but selective REM sleep deprivation potentiates anesthetic effects.[17] REM = rapid eye movement sleep; NREM = non-rapid eye movement sleep.

Effects of Sevoflurane on Sleep Homeostasis

Sevoflurane anesthesia was demonstrated to satisfy NREM sleep debt, which is consistent with the first category. However, sevoflurane showed a more profound effect on NREM homeostasis than was previously demonstrated for propofol—whereas propofol appeared equivalent to *ad libitum* sleep, the effects of sevoflurane were more profound than *ad libitum* sleep. Complete NREM sleep recovery was observed after just 6 h of sevoflurane exposure, which was only achieved after 12 h of *ad libitum* sleep. Of note, the current protocol titrated sevoflurane anesthesia to burst suppression, which differs from the endpoint used for studies of propofol. The endpoint of burst suppression also strongly suggests that the effects of sevoflurane on NREM sleep homeostasis do not derive simply from neurophysiologic similarities to slow-wave sleep.

In contrast, sevoflurane did not appear to be associated with REM sleep homeostasis, because the recovery profile for REM sleep during the postdeprivation and postanesthetic period mirrored one another. In the post–sleep-deprivation recovery period, REM sleep increased to 15.9% from 4.0% in the *ad libitum* group during the first 6 h and then increased to 17.4% (sleep deprivation) from 5.6% (*ad libitum*). Therefore, the total REM sleep during the post–sleep-deprivation recovery period increased consistently for 12 h. In the postanesthetic period, REM sleep in the sevoflurane group increased to 11.6% from 5.6% in the *ad libitum* group during the first 6 h of postanesthetic recovery and then increased to 19.4% (sevoflurane) from 11.8% (*ad libitum*). Again, a consistent increase in the amount of REM sleep could be observed during the postanesthetic recovery period of 12 h. Therefore, it can be argued that REM sleep homeostasis is in a suspended state during inhalational anesthetic exposure. On emergence from anesthesia, the recovery profile in the sevoflurane group follows a circadian pattern that is similar to the sleep-deprivation group (*i.e.*, an increase in the amount of REM sleep for 12 h). This is consistent with the previous findings for isoflurane,[17] suggesting that halogenated ethers in general have a neutral recovery profile for REM sleep and thus fit into the third category described above.

Slow-wave activity or δ power during NREM sleep is a measure of sleep intensity.[20,21] Upon recovery from sleep deprivation, the rats in the current study showed the expected increase in δ power during the immediate 6 h of the postdeprivation period, after which the δ power decreased and stayed below the *ad libitum* sleep levels ("negative rebound") (fig. 5). The observed initial increase in the δ power, and the subsequent negative rebound is consistent with the previously published literature.[20–22] The analysis of δ power during the postanesthetic period showed that the sevoflurane exposure to the sleep-deprived rats attenuated the initial increase in δ power, which otherwise is observed immediately after sleep deprivation. Further, δ power during the postanesthetic period stayed below the *ad libitum* sleep levels, which mirrors the time course of the δ power recovery from sleep deprivation under *ad libitum* sleep conditions (no anesthetic). Therefore, along with the absence of rebound NREM sleep in the postanesthetic period, the recovery profile of δ power confirms that the sevoflurane treatment provided permissive conditions for the completion of homeostatic recovery of NREM sleep. It could be argued that the anesthetic exposure simply suppressed the deeper slow wave activity during the 18-h postanesthetic recovery period. However, there was no observed NREM sleep rebound during this period, but REM sleep rebound was clearly observed during the initial 12 h of the postanesthetic recovery period. A more consistent sleep pattern showing the circadian variations could have been observed had we continued the sleep–wake recordings for more than 24 h postdeprivation, which can be construed as a limitation to the study.

Sleep Deprivation and Anesthetic Potentiation

We observed that sleep deprivation decreased the time to LORR induced with sevoflurane. A similar effect has been previously shown for propofol and isoflurane.[15] Sevoflurane decreases cortical acetylcholine,[23] a correlate of behavioral and electroencephalographic arousal,[2] and sleep deprivation is associated with an increase in adenosine concentration in the basal forebrain.[24] Interestingly, it was recently shown that adenosine in the prefrontal cortex decreases the local acetylcholine release and electroencephalographic arousal.[25] Therefore, increased brain adenosine levels during sleep deprivation might exert a synergistic effect with sevoflurane to decrease the time to loss of consciousness, as indicated by the LORR. The role of adenosine in reducing the time to loss of consciousness is also strengthened by reports that (1) intra-

venous adenosine in mice reduced the time to propofol-induced LORR[26] and (2) aminophylline, an adenosine antagonist, increased the time required to induce loss of consciousness in human subjects.[27] Further, prior treatment of sleep-deprived rats with systemic and/or local administration of adenosine antagonist into the basal forebrain prolonged the time to LORR and reduced the postanesthetic recovery time.[28]

Methodologic Issues

Sevoflurane has been associated with epileptiform discharge, and the 6-h sevoflurane exposure in this study does raise the possibility of persistent behavioral or electroencephalographic effects during the postanesthetic period. However, animal studies done with a much higher sevoflurane concentrations (up to 5.0%) failed to show any behavioral or electroencephalographic evidence of seizure activity during anesthesia[29] and did not find evidence for any postanesthetic effects on the electroencephalogram during any of the sleep–wake states.[30] In addition, sevoflurane exposure decreases cortical acetylcholine levels, which revert to the preanesthetic levels after the cessation of sevoflurane treatment, thereby further arguing against the possibility of any persistent effects of sevoflurane exposure on the electroencephalogram.[23] In conformity with the existing literature, we also did not observe any behavioral or electrophysiologic manifestation of epileptiform activity in any of the animals either during the 6-h anesthetic treatment or during the postanesthetic recording period. The autoscored sleep–wake data were subjected to random manual checks by blinded experimenters. Further, it would be reasonable to expect that a subject emerging from a 6-h anesthetic titrated to burst suppression may show signs of slow electroencephalographic activity, as observed during NREM sleep. However, the animals in our study did not show any such electroencephalographic signs. Collectively, it is improbable that sevoflurane exposure *per se* affected the recovery profile of sleep–wake states.

Scientific and Clinical Significance

The relationship of general anesthesia and sleep homeostasis provides a composite picture of the interactions of anesthetics with sleep–wake centers in the brain. As such, the differential effects of inhalational agents on NREM and REM sleep, as well as the differences compared with propofol, suggest that there is no indiscriminate "sleep-anesthesia" connection. Based on our findings, interfaces of sleep mechanisms and anesthetic mechanisms are likely to be agent and state specific (fig. 6). Further studies could explore differential effects on the nuclei controlling sleep–wake states that might account for the behavioral observations of this study.

Clinically, these data beg the question of which agents would best satisfy sleep homeostasis in the perioperative period. Patients with untreated obstructive sleep apnea may present for surgery with significant sleep fragmentation, and the subsequent REM sleep rebound phenomenon during recovery may be associated with airway obstruction and hypoxemia.[31,32] Our data suggest that different anesthetics may satisfy different components of sleep debt in the perioperative setting. Further studies are required to see whether the differential effects of intravenous and inhalational anesthetics on sleep homeostasis have clinical relevance in the perioperative period, which has the additional influences of surgical stress and the effects of opioids.

Conclusion

Sevoflurane anesthesia has differential effects on NREM and REM sleep homeostasis, and deep sevoflurane anesthesia allows the accelerated homeostatic recovery of NREM sleep. Further, the current data support our previous suggestion[17] that inhalational agents do not satisfy the homeostatic need for REM sleep. Ongoing studies are being conducted with a uniform sleep-deprivation paradigm and a uniform electroencephalographic anesthetic endpoint to better compare the effects of different agents on sleep homeostasis. Further study in the growing population of patients with sleep disorders, such as obstructive sleep apnea, is also required to assess the clinical significance of these differences in the perioperative setting.

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