Rapid Eye Movement Sleep Debt Accrues in Mice Exposed to Volatile Anesthetics

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ABSTRACT

Background: General anesthesia has been likened to a state in which anesthetized subjects are locked out of access to both rapid eye movement (REM) sleep and wakefulness. Were this true for all anesthetics, a significant REM rebound after anesthetic exposure might be expected. However, for the intravenous anesthetic propofol, studies demonstrate that no sleep debt accrues. Moreover, preexisting sleep debts dissipate during propofol anesthesia. To determine whether these effects are specific to propofol or are typical of volatile anesthetics, the authors tested the hypothesis that REM sleep debt would accrue in rodents anesthetized with volatile anesthetics.

Methods: Electroencephalographic and electromyographic electrodes were implanted in 10 mice. After 9–11 days of recovery and habituation to a 12 h:12 h light-dark cycle, baseline states of wakefulness, nonrapid eye movement sleep, and REM sleep were recorded in mice exposed to 6 h of an oxygen control and on separate days to 6 h of isoflurane, sevoflurane, or halothane in oxygen. All exposures were conducted at the onset of light.

Results: Mice in all three anesthetized groups exhibited a significant doubling of REM sleep during the first 6 h of the dark phase of the circadian schedule, whereas only mice exposed to halothane displayed a significant increase in nonrapid eye movement sleep that peaked at 152% of baseline.

Conclusion: REM sleep rebound after exposure to volatile anesthetics suggests that these volatile anesthetics do not fully substitute for natural sleep. This result contrasts with the published actions of propofol for which no REM sleep rebound occurred.

What We Already Know about This Topic

- Administration of propofol to rodents satisfies the need for both rapid eye movement (REM) and non-REM sleep

What This Article Tells Us That Is New

- In contrast with published actions of propofol, for which no REM sleep rebound occurred, REM sleep rebound occurs after exposure to volatile anesthetics in rodents, suggesting that volatile anesthetics do not fully substitute for natural sleep

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APPROXIMATELY two decades ago, the call was issued for multidisciplinary studies to address the mechanisms through which anesthetics produce a state of unconsciousness.[1,2] The state of general anesthesia produced by many drugs shares striking similarities with that of nonrapid eye movement (NREM) sleep.[3] During unconsciousness accompanying both states there is a breakdown in effective cortical communication,[4,5] the appearance of slow waves that share a similar origin and directionality,[6] and asymmetry of directional information transfer.[7–9] Functional imaging studies demonstrate that activity in the default mode network is changed with the unconsciousness accompanying both NREM sleep and general anesthesia, although the specific adaptations in default mode network admittedly are not identical,[10,11] leading to questions about the true degree of convergence between sleep and anesthetic-induced hypnosis.[12] Nonetheless, neurophysiologic similarities extend beyond the cortex to subcortical systems as well, where imaging studies have revealed a deactivation of the thalamus and midbrain reticular formation coincident with loss of consciousness.[13–16] Moreover, animal studies show that sleep deprivation potentiates the hypnotic potency of anesthetics[17,18] as does administration of endogenous somnogens.[19,20] Although the states of anesthesia and NREM sleep are clearly not the same, anesthetics impair the neuronal circuits that promote endogenous arousal.[21–24] This has led to the speculation that the brains of anesthetized subjects may be locked into a NREM-like state while being mutually excluded from accessing rapid eye movement (REM) sleep and wakefulness.[13,25] If true, then time spent in anesthesia might fulfill NREM sleep requirements, but one might expect a REM rebound upon termination of the anesthetic exposure as the homeostatic drive for REM sleep accrues.[26]

However, seminal studies conducted with the intravenous anesthetic propofol refute the preceding hypothesis. In rats, preexisting sleep debts accumulated over the previous 24 h dissipate identically under propofol anesthesia as they would have during recovery sleep.[27] Moreover, unlike true sleep deprivation, when propofol anesthesia is induced at the onset of the rest cycle in rodents and maintained for 12 h, neither REM nor NREM sleep debts accrue.[28] With respect to subsequent sleep, the degree to which the state of anesthesia produced by propofol may be reflective of other anesthetics remains unknown. It has become increasingly clear that many agent-specific differences exist among anesthetics, including diversity in the neuronal mechanisms through which individual drugs produce the anesthetic state.[23,29–34]

Contradictory evidence with volatile anesthetics in multiple species, including humans, suggests that anesthetics may not substitute for natural NREM and REM sleep.[35–39] However, detailed studies of sleep and wakefulness after anesthetic exposure as defined by incorporating electroencephalographic-based analysis of sleep and its microarchitecture are currently lacking. Moreover, comparative studies examining the isolated effects of volatile anesthetics on sleep in the absence of surgical insults are also needed. We therefore conducted the following studies in mice to test the hypothesis that REM but not NREM sleep debts would accrue during exposure to volatile anesthetics and that unlike propofol, the volatile anesthetics would not fully substitute for endogenous REM and NREM sleep. In addition to their potential clinical application, these studies also suggest that important differences exist in the ways that volatile anesthetics and propofol interact with endogenous arousal circuits.

Materials and Methods

Animals

All studies were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania (Philadelphia, PA) and were conducted in accordance with the National Institutes of Health guidelines.†† Adult C57BL6/J male mice (Jackson Labs, Bar Harbor, ME) aged 8–12 weeks were placed on a 12 h light-dark cycle and given food and water *ad libidum*. Lights were turned on at 7 AM—Zeitgeber time (ZT) 0 and turned off at 7 PM, ZT12.

Surgery

Wild-type mice (n = 10) were surgically implanted with four electroencephalographic and two electromyographic electrodes according to previous published protocols[40–42] with the following modifications. Briefly, anesthesia was induced with 2.5% isoflurane and maintained with 1.5–2.0% isoflurane, titrated to the absence of response after tail and foot pinch. Animals were placed into a digital stereotactic frame (David Kopf Instruments, Tujunga, CA) and warmed on a heating pad to maintain core body temperature at 36 ± 1°C. Aseptic technique was undertaken. The skin was cleaned with betadine. A midline incision over the scalp exposed the skull to permit drilling of four burr holes at ± 2 mm lateral to Bregma and 1 mm rostral overlying primary motor cortex and ± 2 mm lateral to Bregma and 3 mm caudal to Bregma overlying primary visual cortex. A lightweight socket headpiece, created by soldering 28-gauge Teflon insulted silver wires (A-M Systems, Sequim, WA) onto a six-pin straight dual-row personal computer board connector (Allied Electronics, Fort Worth, TX), was inserted with one wire cemented into each burr hole to create four epidural electroencephalographic leads. The remaining two wires were placed into the nuchal musculature to create electromyographic leads. The entire headpiece was secured to the skull with dental cement (A-M Systems).

Mice were allowed a minimum of 7 days of recovery after surgery. During the recovery period, mice were singly housed and acclimated to the recording chamber, a custom- designed cylindrical cage with a diameter of 7.625 inches, a height of 6.5 inches, and a volume of 4.9 l, which also served as their new home cage. The recording chamber could be

†† Available at: http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf. Accessed May 10, 2011.

sealed to become gas tight and had fresh gas inlet and outlet ports, along with two luer adaptors at the base for sampling and returning the recording chamber gas mixture. Temperature in the cages was kept at 25 ± 2°C. After recovery, the implanted socket headpieces were connected to the recording apparatus *via* a flexible tethering cable (Plastics One, Roanoke, VA) that allowed the mice to habituate for another 2–4 days before baseline sleep-wake recordings.

Physiologic Recording of Behavioral State

Polysomnographic recordings of murine electroencephalographic and electromyographic waveforms were amplified and sampled at 200 Hz using an MP150 recording system (Biopac Systems Inc, Goleta, CA). High- and low-pass filters were set at 1.0 Hz and 100 Hz for electroencephalogram and 1.0 Hz and 500 Hz for electromyogram recordings, respectively. Data were sent to an eMac computer running AcqKnowledge 3.9.1 (Biopac Systems Inc). Data were converted for analysis with Somnologica Studio version 3.2.1 with Rodent Sleep Scoring Module (EMBLA, Broomfield, CO). States of REM, NREM, and wakefulness in mice were determined by an experienced scorer and verified by a board-certified sleep medicine physician (E.B.F.) on the basis of the predominant state within each 10-s epoch. Both scorers were blinded to the experimental condition. There was a 91.7% overall agreement between double-scored recordings of sleep and wakefulness in keeping with published studies of both human-human and human-computer concordance.[43] Wakefulness was recognized by the presence of low amplitude, fast frequency, desynchronized electroencephalogram combined with increased motor tone. NREM sleep was recognized by the presence of high amplitude, slow frequency electroencephalographic activity with reduced motor tone relative to wake. REM sleep was recognized by the presence of low-amplitude, fast frequency, desynchronized electroencephalogram with a peak in θ frequency, together with minimal to absent motor tone.[40,44]

Sleep microstructure was distinguished by determining the number of wake bouts, NREM bouts, and REM bouts as well as the average bout length for each sleep state. Bout numbers were counted as each continuous episode of the sleep state. Bout length was calculated by the amount of time spent in separate bouts. Spectral analysis was performed using the Somnologica Science software application. Electroencephalographic data were fast Fourier transformed in 10-s epochs. The δ power (1.0–4.0 Hz) was computed for each NREM epoch and averaged over all NREM sleep epochs within three temporal periods: L2, corresponding to the second portion of the light cycle (ZT7–12); D1, corresponding to the first portion of the dark cycle (ZT12–18); and D2, corresponding to the second portion of the dark cycle (ZT18–24). δ power is reported as a percentage of raw NREM δ power at baseline to raw NREM δ power after anesthetic exposure. Because each mouse was exposed to all conditions (baseline, sevoflurane, isoflurane, and halothane), a decision was made not to normalize total power, but rather to score and report absolute power at each frequency in 0.2-Hz bins.

Experimental Protocol

Baseline recordings of wakefulness, NREM, and REM sleep in instrumented mice were conducted in all mice before anesthetic exposure while flowing 100% oxygen through the gas-tight chambers for 6 h beginning at the onset of the light cycle, ZT0, to simulate conditions on the anesthetic day. Three days after obtaining baseline data, mice were exposed to a volatile anesthetic for 6 h beginning at ZT0, which is coincident with their rest period. During anesthetic challenges fresh gas flows were set to 1 l/min with sevoflurane 2.4%, isoflurane 1.2%, or halothane 1.0%, corresponding to 1.2–1.3 times the ED_{50} for loss of righting reflex in C57BL/6 mice, respectively yielding burst suppression ratios of 4.59% ± 4.19%, 11.74% ± 8.80%, and 2.29% ± 3.85% compared with a baseline burst suppression ratio of 0.02% ± 0.03% in the absence of any anesthetic. Burst suppression ratios were determined using epoch length of 60 s, a voltage cutoff of 15 μV, and an isoelectric duration of 500 ms. Burst suppression ratios were averaged across the entire 6-h exposure for each animal and then averaged across animals. To promote euthermia, each recording chamber was actively heated by partially submerging it inside a water bath set to 37°C, which we have previously demonstrated prevents development of hypothermia in mice exposed to anesthetics.[45,46] At the end of the 6-h anesthetic exposure, the anesthetic was discontinued, but fresh oxygen gas continued to flow for an additional 30 min. Fresh food and water were then returned to the mice, and the chamber lids were reopened to room air. Scoring of NREM sleep, REM sleep, and wakefulness began during the second portion of the light cycle at ZT7 and continued to ZT24 (fig. 1). Mice were given 3–5 days between repeated anesthetic exposures. The first cohort of mice (n = 6) was exposed to isoflurane, sevoflurane, and then halothane. The second cohort of mice (n = 4) was exposed to sevoflurane, isoflurane, and then halothane. Halothane was deliberately chosen to be the last anesthetic based on a published report of cross-tolerance.[47]

Statistical Analysis

The amount of time spent awake, in NREM sleep, and in REM sleep was calculated in three blocks covering the second portion of the light cycle (L2) and both 6-h portions of the dark cycle (D1 and D2). Data are presented as mean ± SE. The data were analyzed with a two-way mixed model repeated measures ANOVA of arousal state as a function of Treatment (baseline, sevoflurane, isoflurane, and halothane) and Time (L2, D1, and D2) using a random effects model for subjects with Prism 4.0c (GraphPad Software, Inc, San Diego, CA) and JMP version 8.0 (SAS Institute, Cary, NC). A Bonferroni correction was applied to all *post hoc* comparisons. Interactions with Treatment and Time were considered

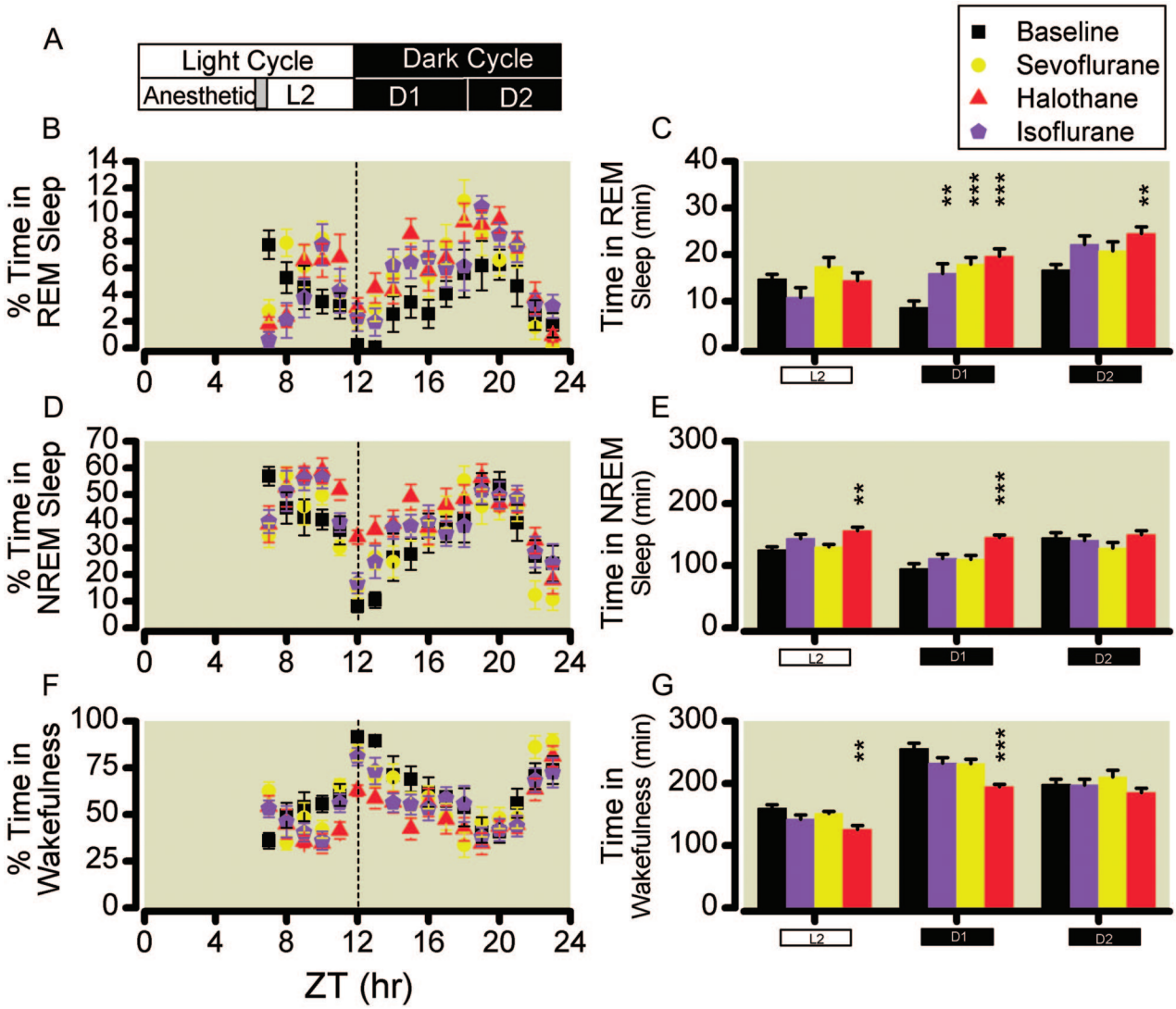

Fig. 1. States of wakefulness, NREM sleep, and REM sleep at baseline and following anesthetic exposure. (*A*) Experimental scheme in which general anesthesia is induced at the beginning of the light cycle and arousal states are recorded over the second portion of the light cycle (L2), along with the first (D1) and second (D2) portions of the dark cycle. The *gray bar* indicates the time given for emergence before recordings of sleep and wakefulness. Percentage of time spent in (*B*) REM sleep, (*D*) NREM sleep, and (*F*) wakefulness each hour as a function of time at baseline (*black*) and after exposure to isoflurane (*purple*), sevoflurane (*yellow*), or halothane (*red*). Total times spent in (*C*) REM sleep, (*E*) NREM sleep, or (*G*) wakefulness over L2, D1, and D2. ** $P < 0.01$ and *** $P < 0.001$ relative to corresponding baseline totals, respectively, by Bonferroni correction *post hoc* analysis of two-way ANOVA. NREM = nonrapid eye movement; REM = rapid eye movement; ZT = zeitgeber time.

and included only when statistically significant. Residuals were analyzed and checked for within subjects' correlations and for constant variation among groups. We observed no significant departures from these assumptions, except where explicitly noted in the text.

The bout lengths for wakefulness, NREM sleep, and REM sleep were averaged to yield the mean bout length of each state in the given time frame. All values are shown as mean ± SE. Data were analyzed with a mixed model repeat measure two-way ANOVA with Bonferroni correction applied for *post hoc* comparisons. In all cases two-tailed *P* values less than 0.05 were considered to be statistically significant.

Results

Exposure to Volatile Anesthetics Causes a Delayed REM Rebound

Using continuous recordings of the electroencephalogram and electromyogram, we measured levels of wakefulness and NREM and REM sleep in wild-type C57BL6/J mice exposed to a general anesthetic for 6 h during the first half of the rest period. Mice exposed to hypnotic doses of sevoflurane, isoflurane, and halothane all exhibited an increase in REM sleep compared with baseline preexposure levels (fig. 1, B and E; $F_{3,99} = 8.39$, $P < 0.0001$). This rebound was time dependent and did not manifest until D1, the onset of the

dark cycle ($F_{2,99} = 18.44$, $P < 0.0001$). Moreover, the repeated measure two-way ANOVA confirmed a significant interaction between the two factors: treatment x time ($F_{6,99} = 3.36$, $P = 0.0046$). After halothane exposure, the REM rebound persisted throughout the entire second half of the dark cycle (fig. 1E).

Exposure to Sevoflurane or Isoflurane Does Not Alter Subsequent Levels of Wakefulness or NREM Sleep, but Exposure to Halothane Does

Previous exposure to isoflurane or sevoflurane did not significantly alter ensuing amounts of NREM sleep or wakefulness. However, previous exposure to halothane was associated with a significant increase in NREM sleep during both the light and first half of the dark periods (fig. 1, C and F). Repeated measure two-way ANOVA confirmed a significant effect of both treatment ($F_{3,99} = 11.46$, $P < 0.0001$) and time ($F_{2,99} = 17.15$, $P < 0.0001$) with a significant interaction of the factors ($F_{6,99} = 2.21$, $P = 0.048$). Increased NREM sleep after general anesthesia with halothane was accompanied by a corresponding significant decrease in wakefulness displaying identical temporal dependency as the NREM changes (fig. 1, D and G). Once again, during wakefulness our ANOVA model revealed a significant effect of both the treatment ($F_{3,99} = 12.91$, $P < 0.0001$) and time ($F_{2,99} = 125.95$, $P < 0.0001$) with no significant interaction of the factors ($F_{6,99} = 2.00$, $P = 0.07$).

Agent-specific Significant Changes in Sleep Architecture

After anesthetic exposure, the underlying structure of sleep-wake cycles exhibited significant changes. As shown in figure 2, anesthetic treatment significantly altered REM bout length ($F_{3,99} = 6.22$, $P = 0.0007$) and number ($F_{3,99} = 5.11$, $P = 0.0025$). Significant temporal effects on REM bout length ($F_{2,99} = 4.51$, $P = 0.0133$) and number ($F_{2,99} = 5.14$, $P = 0.0075$) were also evident. Initial significant daytime increases in REM bout length after exposure to isoflurane and sevoflurane were offset by decreases in the number of REM bouts, whereas halothane had no initial effect on daytime REM sleep structure. When the active phase begins at lights out, ZT12, a nonsignificant increase in REM bout number for isoflurane and sevoflurane or with the significant increase in REM bout number after halothane exposure (fig. 2D) multiplied with nonsignificant changes in the halothane group REM bout length (fig. 2A) to yield a profoundly significant near-doubling of total REM time in the first 6 h of the dark cycle (fig. 1). In the second half of the night, there were no significant changes in REM bout length or its average duration, yet the product of increased bout length and number continued to cause a significant REM rebound only for halothane (fig. 1).

Diversity of the interaction between volatile anesthetics and the neural systems regulating sleep and wakefulness is supported by effects on NREM sleep architecture. As shown in fig. 2, B and E, anesthetic treatment significantly altered NREM sleep bout length ($F_{3,99} = 6.77$, $P = 0.0003$) and number ($F_{3,99} = 5.86$, $P = 0.001$). However, time appeared to affect NREM bout number only ($F_{2,99} = 7.20$, $P = 0.0012$) without affecting NREM bout length ($F_{2,99} = 0.36$, $P = 0.70$). There were no significant interactions between the bout length and anesthetic treatment factors. Compared with preexposure baseline data, 6-h isoflurane exposure significantly shortened the average NREM bout duration but correspondingly increased the number of NREM bouts. Conversely, 6-h halothane exposure led only to an initial significant increase in the length of NREM bouts (fig. 2, B and E). There were no significant changes in the architecture of NREM sleep after exposure to sevoflurane.

With respect to baseline wakefulness, anesthetic drug treatment significantly altered both average wake bout duration ($F_{3,99} = 9.47$, $P < 0.0001$) as well as the number of waking bouts ($F_{3,99} = 6.04$, $P = 0.0008$). Effects of time on the structure of waking were also highly significant for both bout length ($F_{2,99} = 26.72$, $P < 0.0001$) and number of waking bouts ($F_{2,99} = 6.33$, $P = 0.0026$) with a significant interaction of treatment and time occurring only for average bout duration ($F_{6,99} = 3.77$, $P = 0.0023$), but not for number of waking bouts ($F_{6,99} = 0.92$, $P = 0.48$). Exposure to 6 h of volatile anesthetics during the first half of the rest phase caused a subsequent significant decrease in the average duration of wake bouts during the first half of the active period, D1. This is consistent with a fragmenting of postanesthetic arousal state maintenance with a significant degradation in the stability of wake state (fig. 2C). This net decrease in wake bout length was offset by increases in the number of wake bouts during D1 (fig. 2F). In the case of isoflurane, wake bout number was significantly increased during both halves of the dark period.

Latency to Enter REM Sleep Is Significantly Reduced During the Active Period After Exposure to Sevoflurane, Isoflurane, or Halothane

Anesthetic treatment significantly changed sleep latency ($F_{1,63} = 3.58$, $P = 0.0185$). Moreover, there was a significant interaction between the time and treatment factors ($F_{3,63} = 13.62$, $P < 0.0001$). In comparison with the preanesthetic exposure baseline, mice exhibited a significantly shorter latency to entering REM sleep, consistent with an increased homeostatic pressure to enter REM sleep. REM sleep latency in D1 decreased more than threefold from more than 3 h to less than 1 h for all three anesthetic agents and was highly significant by *post hoc* Bonferroni correction analysis as shown in figure 3. Immediately after the anesthetic exposure through the end of the light cycle, all mice exhibited an increased latency to enter REM sleep, which reached significance only for isoflurane. An analysis of the residuals indicated that variance increased with latency. Nonetheless, the results are nearly identical ($F_{3,63} = 13.37$, $P < 0.0001$) when a variance stabilizing square root transformation is applied to the response.

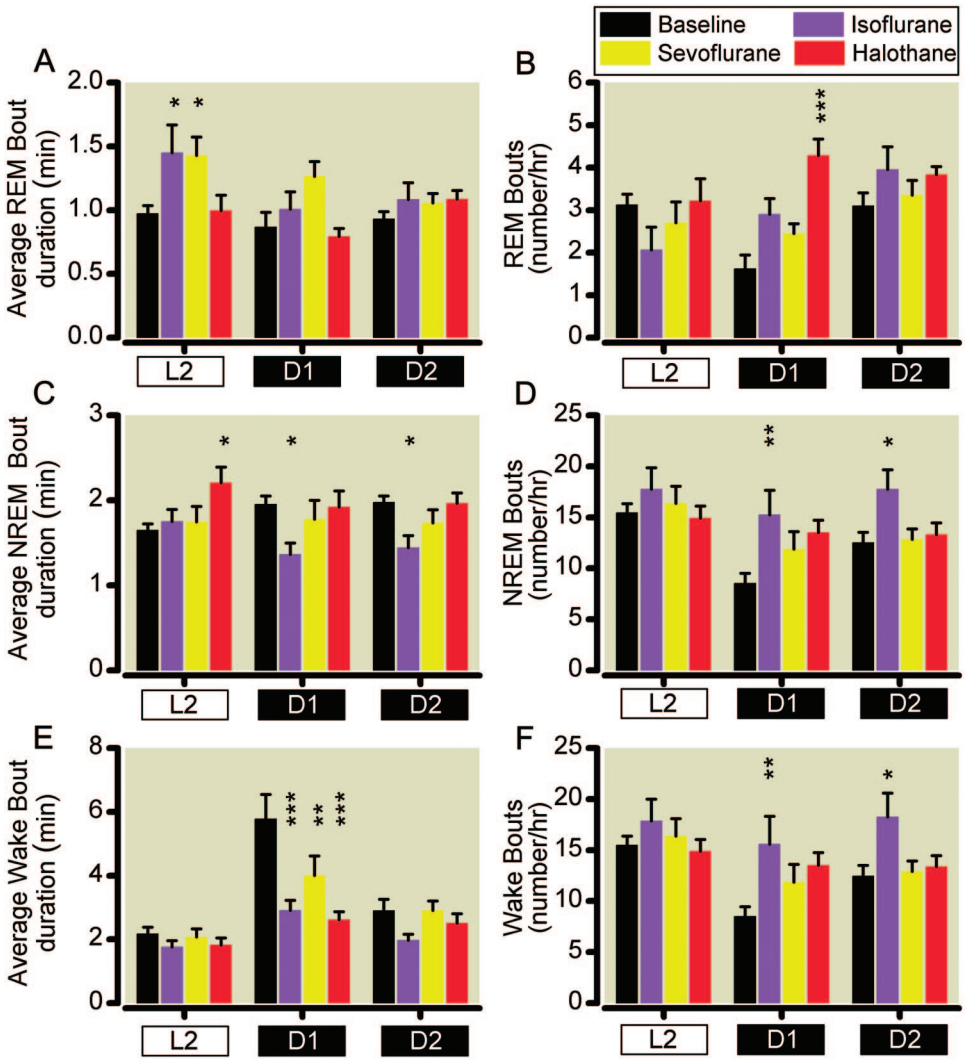

Fig. 2. Sleep architecture at baseline and after volatile anesthetic exposure. Average length for each bout of (*A*) REM, (*C*) NREM, and (*E*) wakefulness along with the number of bouts for (*B*) REM, (*D*) NREM, and (*F*) wakefulness at baseline (*black*), and after 6 h of exposure to sevoflurane (*yellow*), isoflurane (*purple*), or halothane (*red*) in L2, the second portion of the light cycle; D1, the first portion of the dark cycle; and D2, the second portion of the dark cycle. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ relative to corresponding baseline totals, respectively, by Bonferroni correction *post hoc* analysis of two-way ANOVA. NREM = nonrapid eye movement; REM = rapid eye movement.

Latency to Enter NREM Sleep Is Significantly Reduced During the Active Period Only After Exposure to Halothane

NREM sleep latency was significantly changed by time ($F_{1,63} = 7.32$, $P = 0.009$). Moreover, there was a significant interaction between time and treatment ($F_{3,63} = 3.09$, $P = 0.03$). *Post hoc* Bonferroni correction testing revealed a significant reduction in NREM sleep latency only after halothane exposure (fig. 3).

Homeostatic Pressure to Enter Deep State of NREM Sleep Is Not Increased After Exposure to Sevoflurane, Isoflurane, or Halothane

Unlike general anesthesia, sleep is homeostatically regulated. After sleep deprivation, there is an increased propensity for the deprived subject to enter the deep, restorative phases of slow-wave NREM sleep, which is characterized by highly synchronized, large amplitude waveforms observed on the electroencephalogram with a frequency less than 4 Hz in the δ range. This increased homeostatic propensity or drive to sleep is manifested by an increased amount of slow-wave sleep and is measured by performing a Fourier transformation on the electroencephalogram and computing the power in the frequency bins less than 4 Hz (δ power).[48] If states of general anesthesia elicited by volatile anesthetic exposure physiologically deprived subjects of NREM sleep, then relative to baseline, a significant increase in NREM δ power should be induced after anesthetic exposure. However, if general anesthesia fulfils the requirements of NREM sleep no increases in δ power would be expected. After 6 h of volatile anesthetic exposure given at the onset of the rest phase, we

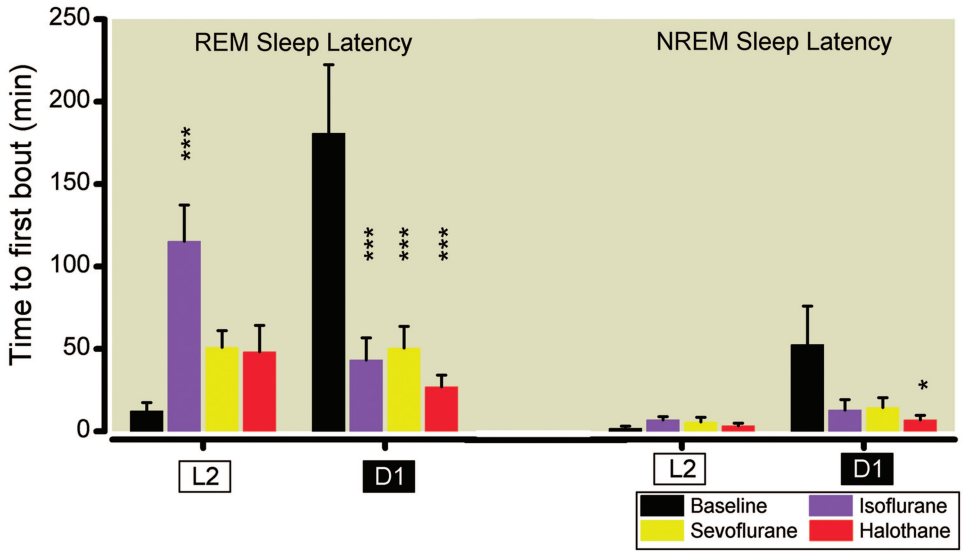

Fig. 3. Sleep latency at baseline and after volatile anesthetic exposure. Time until the first bout of REM and NREM sleep following emergence in L2, the second portion of the light cycle and D1, the first portion of the dark cycle. * $P < 0.05$ and *** $P < 0.001$ relative to baseline, respectively, by Bonferroni correction *post hoc* analysis of two-way ANOVA. NREM = nonrapid eye movement; REM = rapid eye movement.

failed to uncover any ensuing significant increase in δ power (fig. 4). Instead, in comparison with baseline, NREM sleep δ power was actually reduced following volatile anesthetic exposure. Two-way mixed model ANOVA revealed a significant effect of anesthetic treatment on δ power ($F_{3,99} = 9.50$, $P < 0.0001$). Meanwhile, there was no significant effect of time ($F_{2,99} = 0.57$, $P = 0.56$) and no significant interaction between time and treatment ($F_{6,99} = 1.85$, $P = 0.097$) upon δ power in NREM sleep after volatile anesthetic exposure. Nevertheless, we observed that the largest reduction in δ power occurred at the beginning of the active phase where previous isoflurane exposure significantly reduced homeostatic drive to sleep. Detailed spectral decomposition of the electroencephalographic power in all NREM sleep epochs as a function of time (fig. 5) also demonstrated that relative to baseline, there was reduced power in slow (less than 4 Hz) frequency bins that was most pronounced during D1 and D2.

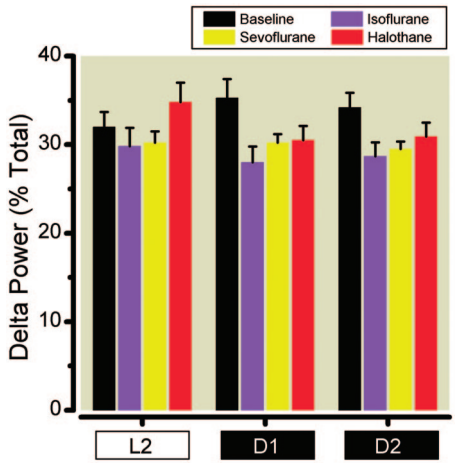

Fig. 4. δ power (1.0–4.0 Hz) at baseline (*black*), and after 6 h of exposure to sevoflurane (*yellow*), isoflurane (*purple*), or halothane (*red*) in L2, the second portion of the light cycle; D1, the first portion of the dark cycle; and D2, the second portion of the dark cycle.

Discussion

Herein we demonstrate that the state of general anesthesia does not fully substitute for natural sleep. In contrast with propofol, a 6-h general anesthetic initiated and maintained by the volatile anesthetics sevoflurane, isoflurane, or halothane does indeed incur a REM sleep deficit, whereas only halothane accrues an additional NREM sleep debt. Each volatile anesthetic fragmented subsequent wakefulness during the animal's ensuing active period. This was most severe for halothane, which also significantly reduced the total duration of wakefulness.

The response to sleep-depriving stimuli depends on the circadian phase in which it is administered.[49] Our anesthetic delivery was specifically chosen to coincide with the onset of the light cycle—the rodent rest phase. We reasoned that should the anesthetic state function in an analogous manner to a "sleep deprivation" by precluding subjects from activating the neural circuits necessary for NREM and/or REM sleep, then the ensuing propensity to sleep, also known as the homeostatic pressure, would be maximized by delivering the anesthetic at the end of the waking period.

One readily detected manifestation of sleep deprivation is the ensuing rebound when deprived subjects are presented with the opportunity for recovery sleep.[50] For NREM sleep, this recovery may manifest as increased total NREM sleep duration; increased intensity of NREM sleep, indicated by an increase in NREM δ power; increased consolidation of

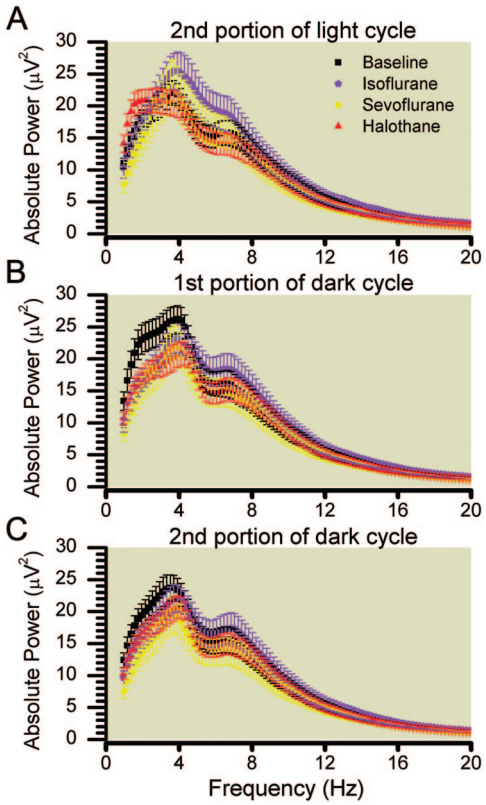

Fig. 5. Average spectral power during nonrapid eye movement sleep at baseline (*black*), and after 6 h of exposure to sevoflurane (*yellow*), isoflurane (*purple*), or halothane (*red*) over (*A*) L2, the second portion of the light cycle; (*B*) D1, the first portion of the dark cycle; and (*C*) D2, the second portion of the dark cycle.

NREM sleep bouts; and decreased latency to enter NREM sleep. Conversely, for REM sleep there is no known means to increase REM intensity or quality within a single bout, hence it has been proposed that REM sleep deficits can only be compensated by a shorter latency to the first REM bout and by increased total REM sleep time.[50]

As with a 6-h sleep deprivation produced by gentle handling or wheel running,[51,52] a 6-h volatile anesthetic exposure also caused a delayed REM rebound that was expressed during the first half of the mouse's active phase for sevoflurane and isoflurane and for the entire active phase for halothane. Consistent with the increased REM pressure during the mouse's active phase, we demonstrate a significantly shorter latency to enter REM sleep. The magnitude of REM sleep rebound in the first half of the active phase caused by exposure to isoflurane, sevoflurane, and halothane was 184%, 207%, and 228%, respectively, of the baseline level of REM sleep, and was nearly identical to the 220% seen after 6 h of automated sleep deprivation by wheel running in mice,[52] and was comparable with the rebound after 24 h of total sleep deprivation in rats.[53]

Whereas the REM sleep rebound appeared largely comparable to that expected in rodents after traditional sleep deprivation, NREM sleep after sevoflurane or isoflurane exposure did not increase in total amount, in average NREM sleep bout duration, or in NREM sleep intensity as defined by increased δ power. Although there was a decreased latency to enter NREM sleep in the active phase after isoflurane or sevoflurane exposure, these changes failed to reach significance. Hence, by all criteria, a 6-h exposure to isoflurane and sevoflurane did not induce a NREM rebound. Halothane, which is known to exhibit distinct cortical and subcortical effects from the halogenated ether anesthetics isoflurane and sevoflurane at equipotent doses,[40,54–57] showed a distinct profile with evidence for rebound NREM sleep. After halothane exposure, the total time mice spent in NREM sleep significantly increased both immediately and into the second half of their active phase. This increase was accompanied by an initial increase in NREM sleep bout length in accordance with published results in mice after 6 h of sleep deprivation by gentle handling,[58] and with decreased latency to enter NREM sleep. Similar to other anesthetics,[18,25,27,59] but unlike sleep deprivation,[48] NREM sleep after halothane exposure was not associated with increased δ power.

Cumulatively our findings suggest that sevoflurane and isoflurane may satisfy the homeostatic drive for NREM sleep. Specifically, in the case of isoflurane and sevoflurane, NREM sleep pressure may be discharged by the state of general anesthesia.[18,25,59] However, like halothane and unlike propofol, sevoflurane and isoflurane do not satisfy the homeostatic drive for REM sleep. This conclusion is supported by additional studies in rodents in which sleep-deprived rats anesthetized with isoflurane or sevoflurane were unable to dissipate preexisting REM sleep deficits during exposure to the volatile anesthetic.[18,25]

Results obtained herein suggest that the brain is still able to track time elapsed under general anesthesia.[60] Our results with three distinct volatile anesthetics suggest that REM and NREM homeostatic tracking mechanisms could be distinct. Were the homeostatic markers of NREM and REM sleep dependent only on synaptic activity, an increased probability of synaptic failure in the presence of general anesthetics could erase information stored by the homeostat. Alternatively, the homeostatic encoding of accrued sleep debts could be stored purely by a neurochemical signal that amasses with neuronal activity, but exists in an activity-independent, stable form. In this latter case, dysfunction in synaptic neurotransmission by volatile anesthetics would not be sufficient to reset the homeostat. Such a possibility would lead to a permissive state in which the homeostat might be frozen in place but still able to track preexisting debts, or unaffected by the presence of the anesthetics and able to run as if no drug were present. Data by Pal et al.[18] and Mashour et al.[25] demonstrate the latter possibility. Our study, in which no sleep debts were accrued before anesthetic exposure, allows further insight into homeostatic functioning during anesthesia. Finding a distinct interaction of anesthetic drugs with neural circuits regulating arousal is consistent with agent-specific distinct interactions with the homeostat. The absence of NREM rebound after

sevoflurane or isoflurane exposure implies either a timekeeping failure or that the anesthetic state has partially substituted for NREM sleep. Halothane anesthesia would appear fundamentally different by not satisfying homeostatic need for NREM or REM sleep. As additional knowledge is gained about the interactions of distinct anesthetics with the REM and NREM homeostats, it might be possible to use general anesthetics to decode the anatomic loci and intrinsic regulation underlying sleep need.

Study Limitations

Several methodologic issues merit consideration when examining our results. To minimize experimental variability, mice were exposed in parallel to identical gas flow conditions while euthermia was preserved. Groups of four or six mice were treated simultaneously. This design prevented true randomization of anesthetic exposure, potentially biasing our results based on the order of volatile anesthetic presentation. This flaw may be mitigated as short-term tolerance to volatile anesthetics does not occur, which makes cross-tolerance also doubtful.[47,61] We did not acquire respiratory data and cannot formally exclude intermittent hypoxia or hypoventilation during anesthetic exposures. We attempted to normalize exposures using equipotent anesthetic concentrations that were 1.2–1.3 times the ED_{50} for loss of righting in mice.[45] Sevoflurane and halothane equipotency was also confirmed by a second anesthetic endpoint—indistinguishable levels of burst suppression. However, with isoflurane we observed a significantly higher incidence of burst suppression. As REM rebound occurred after exposure to all volatile anesthetics and NREM rebound occurred only after halothane exposure, we believe that the depth of anesthesia as defined by burst suppression or potentially by relatively greater isoflurane levels cannot fully explain our findings. Of note, shorter anesthetic exposures may not be sufficient to accrue potential sleep debts.[62]

Conclusion and Implications

In summary, we demonstrate that a 6-h exposure to hypnotic doses of volatile anesthetics incurs a REM sleep deficit in mice, which is followed by a significant REM rebound hours after the cessation of the anesthetic. Such results are consistent with the notion that the brain anesthetized with volatile anesthetic is prevented from accessing the neuronal circuits driving REM sleep, whereas the REM homeostat continues to register the accruing deficit. Although isoflurane and sevoflurane appear to fulfill NREM sleep need, halothane does not. Although extrapolating results from a murine model to humans is fraught with difficulties, previous human studies also demonstrate similar adaptations in sleep following anesthesia. However, these studies either permitted unmonitored daytime napping and measured only nighttime sleep electroencephalographically in healthy volunteers[37] or showed a significant REM sleep rebound, but were conducted in the setting of actual surgery and potentially confounded by pain and polypharmacy.[35,36] Nevertheless, our results in healthy adult mice support the idea of REM rebound after volatile anesthetic exposure and potentially herald important clinical morbidity. REM sleep loss can cause hyperalgesia.[63] Moreover, delayed REM rebound after volatile anesthetic exposures may place patients at risk for episodes of hypoxemia[64] and is especially concerning in patients with obstructive sleep apnea in whom hypoventilation, carbon dioxide retention, oxygen desaturations, and bradyarrhythmias often accompany REM sleep.[65] REM rebound has also been linked to hemodynamic instability, myocardial infarction, stroke, and postoperative delirium.[64] Together with other preclinical animal studies[18,25,27,28,59] our data suggest that important agent-specific differences may exist in the ways that individual general anesthetic drugs affect sleep. Hence, opportunities exist to determine whether the potential disruption of sleep by distinct anesthetics actually heralds heightened morbidity in vulnerable human populations; and whether an agent with a more favorable postanesthetic sleep profile, such as propofol,[27,28] might make a better choice to reduce potential perioperative complications associated with REM sleep rebounds.

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