

## Effects of Anesthesia on the Response to Sleep Deprivation

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**Study Objective:** Slow wave activity (SWA) during NREM sleep is the best characterized marker of sleep homeostasis, and the occurrence of sleep slow waves is necessary to reduce sleep need. Recent evidence suggests that sleep slow waves may mediate several beneficial effects of sleep on performance, from the prevention of cognitive impairments to memory consolidation. However, slow waves are also triggered by low doses of many anesthetics, but very few reports have examined whether anesthesia-mediated slow waves affect the homeostatic regulation of sleep. Moreover, no study has examined how sleep is affected by higher doses of anesthetics, which lead to a predominantly “isoelectric” EEG tracing without slow waves.

**Design:** We studied in rats whether 1 hour of a dose of isoflurane or desflurane able to induce almost continuous slow waves (ISO-sw, DES-sw), and of a dose of desflurane resulting in a predominantly isoelectric EEG (DES-iso) reduces the sleep pressure caused by 4 h of sleep deprivation. Anesthesia was compared to a mock condition in which rats were only anesthetized for 2-3 min.

**Setting:** Basic sleep research laboratory.

**Patients or Participants:** Male WKY rats (n = 31).

**Interventions:** Total sleep deprivation by exposure to novel objects starting at light onset, followed by one hour of anesthesia or mock anesthesia.

**Measurements and Results:** One hour of anesthesia (sw or iso) did not affect either sleep duration or the overall sleep pattern. Anesthesia with ISO-sw or DES-sw, both associated with the occurrence of almost continuous slow waves, reduced the SWA rebound expected following 4 h of sleep deprivation. One hour of anesthesia with DES-iso, associated with isoelectric EEG and few slow waves, also reduced the SWA rebound after sleep deprivation, and did so to an extent similar to that observed after DES-sw. However, in contrast to DES-sw, SWA after DES-iso remained chronically lower than in baseline, resulting in reduced slow wave energy (SWE, SWA × time) for at least 2 days.

**Conclusion:** The blunted SWA rebound after ISO-sw and DES-sw suggests that anesthesia slow waves may substitute for sleep slow waves. The reduced SWA rebound after DES-iso may reflect a pathological condition that results in a chronic decrease in SWA, or may suggest that anesthesia slow waves are not an absolute requirement to discharge sleep pressure.

**Keywords:** Desflurane, isoflurane, rat, slow wave activity

**Citation:** Nelson AB; Faraguna U; Tononi G; Cirelli C. Effects of anesthesia on the response to sleep deprivation. *SLEEP* 2010;33(12):1659-1667.

SLEEP IS HOMEOSTATICALLY REGULATED IN THAT SLEEP NEED INCREASES WITH TIME SPENT AWAKE AND DECREASES DURING SLEEP. THE BEST characterized marker of sleep homeostasis is SWA, the EEG power between 0.5-4.0 Hz during NREM sleep. In birds and mammals NREM SWA reaches an apex at the beginning of the sleep period and decreases with time spent asleep.<sup>1-3</sup> Moreover, staying awake from ~3 to ~24 hours results in progressively higher SWA levels at sleep onset, while naps during the day reduce SWA the following night.<sup>4,5</sup> Usually recorded from the scalp via the EEG, SWA reflects synchronous firing of large neuronal populations that are coordinated by an underlying slow oscillation, the fundamental cellular phenomenon of NREM sleep.<sup>6,7</sup> Increasing evidence suggests that slow waves are more than just an epiphenomenon of NREM sleep. In fact, they can mediate some of sleep's beneficial effects. For instance, intermittent transcranial direct current stimulation during early NREM sleep, which temporarily increased SWA, was found to enhance retention in a paired-associate memory task.<sup>8</sup> Moreover, pharmacological enhancement of slow wave

sleep reduces cognitive impairments associated with sleep restriction.<sup>9,10</sup> Finally, two recent studies showed that the selective suppression of slow waves using acoustic stimuli (without waking up the subjects) prevented post-sleep improvement in performance after visual texture discrimination learning<sup>11</sup> and visuomotor learning.<sup>12</sup> Thus, it seems that slow waves may benefit several aspects of performance, from the prevention of cognitive impairments to memory consolidation.

Much work has been done to characterize the role of slow waves in sleep homeostasis, and it has been demonstrated that the occurrence of slow waves during NREM sleep is necessary to reduce sleep need.<sup>13</sup> However, slow waves are not exclusive to sleep states and, in fact, the slow oscillation was first reported simultaneously in anesthetized cats and sleeping humans.<sup>14</sup> Indeed, many anesthetics used in the clinic, including the volatile anesthetics isoflurane (ISO) and desflurane (DES) and the intravenous agent propofol produce, at relatively low concentrations, a NREM-like EEG activity dominated by slow waves, while higher concentrations lead to an isoelectric tracing which lacks slow waves.<sup>15</sup> However, whether slow waves as induced by anesthetics affect the homeostatic regulation of sleep remains largely unexplored. In humans a 3-h period of low concentration ISO in the morning was found to reduce the percentage of time spent in slow wave sleep during the subsequent night,<sup>16</sup> suggesting that sleep pressure may have been reduced by anesthesia. However, the EEG was not recorded during anesthesia, and thus whether continuous slow waves were indeed present was not confirmed. Moreover, ISO was administered in the morning, when the sleep pressure was presumably low,

A commentary on this article appears in this issue on page 1583.

Submitted for publication January, 2010

Submitted in final revised form July, 2010

Accepted for publication July, 2010

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and 75% of the subjects were allowed to take naps, making the interpretation of the subsequent reduction in slow wave sleep quite difficult. In a more recent study, rats were sleep deprived for 24 h and then anesthetized with propofol for 6 h.<sup>17</sup> After recovery from anesthesia, the duration of NREM and REM sleep decreased, and SWA did not show the rebound normally expected after prolonged sleep loss. However, rats were allowed to recover during the dark phase, when they are normally active. Moreover, because of filter settings, only the high range (2–4 Hz) SWA was analyzed. Several studies found that the SWA rebound after sleep deprivation is either most prominent,<sup>18</sup> longer lasting,<sup>19,20</sup> or even limited to its lowest frequency range.<sup>21</sup> Thus, it is important to assess whether anesthesia affects low range sleep SWA. Finally, a recent study found that 4 h of ISO anesthesia (presumably with slow waves) does not affect the REM sleep rebound after selective REM sleep deprivation for 24 h, but NREM sleep and SWA were not studied.<sup>22</sup>

The goal of this study was to determine whether 1 hour of anesthesia characterized by continuous slow waves reduces the sleep pressure previously created by a period of total sleep deprivation. To this end rats were sleep deprived for 4 h starting at light onset, anesthetized for 1 hour, and then allowed to recover during the light phase, when they normally sleep. Moreover, we wanted to determine whether the effects on recovery sleep differ when the dose of anesthetic is increased to induce isoelectric EEG with few slow waves.

## MATERIALS AND METHODS

### Animals, Surgery, and Recordings

Male WKY rats ( $n = 31$ ; Charles River Laboratories, Wilmington, MA; 250–300 g at time of surgery) were maintained on a 12 h light / 12 h dark cycle (lights on at 10:00; room temperature  $23 \pm 1^\circ\text{C}$ ). Under deep ISO anesthesia (1.5%–2% volume), rats were implanted bilaterally for chronic polysomnographic recordings in the frontal cortex (B, + 2–3 mm; L 2–3 mm) and parietal cortex (B - 3–4 mm; L 3–4 mm). For experiments using ISO ( $n = 14$  rats) bipolar local field potential electrodes (PlasticsOne Inc, Roanoke, VA; Rhodes Medical Instruments, Summerland, CA) were used, while for experiments with DES ( $n = 8$  rats), gold screws were used for EEG recording. Nine additional rats implanted with bipolar local field potential electrodes were used for sleep deprivation experiments without any anesthesia (see Results). LFP bipolar wire electrodes had 1 mm vertical and 0.8 mm horizontal separation between electrode tips, with the shallow lead aimed at cortical layers I–II.

Electrodes were fixed to the skull with dental cement. All animals used for the experiments showed opposite polarity in the EEG signal when superficial and deep electrodes were independently referenced to the cerebellar screw. Gold screws (instead of LFP electrodes) were used for the DES experiments because they provide a signal that is more stable over time, and the study did not require the increased spatial resolution provided by LFP electrodes. The differences in signal between the LFP electrodes and gold screws are not relevant for the findings of this paper and therefore from here onward both LFP and gold screw recordings will be referred to simply as EEG signals. Two stainless-steel wires (diameter, 0.4 mm) inserted into the neck muscles were used to record the electromyogram (EMG).

Immediately after surgery, the animals were individually placed in transparent Plexiglas cages ( $36.5 \times 25 \times 46$  cm), and kept in sound-attenuating recording boxes for the duration of the experiment. At least 8 days were allowed for recovery after surgery, and experiments were started only after the sleep/wake cycle had fully normalized. The rats were connected by means of a flexible cable to a commutator (Airflyte, Bayonne, NJ) and recorded continuously. To habituate the animals to the sleep deprivation procedure (see below), every day beginning the day after surgery, rats were handled and exposed to a novel object between 10:00 and 10:30. Video recordings were performed continuously with infrared cameras (OptiView Technologies, Potomac Falls, VA) and stored in real-time (AVerMedia Technologies, Milpitas, CA; 24 Seven Surveillance Inc, Alhambra, CA). To verify that the animals were fully entrained to the light/dark cycle, cages were equipped with Chronokit activity monitor infrared sensors (Stanford Chronokit; Stanford Software Systems, Santa Cruz, CA). All animal procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AAALAC.

### Data Acquisition

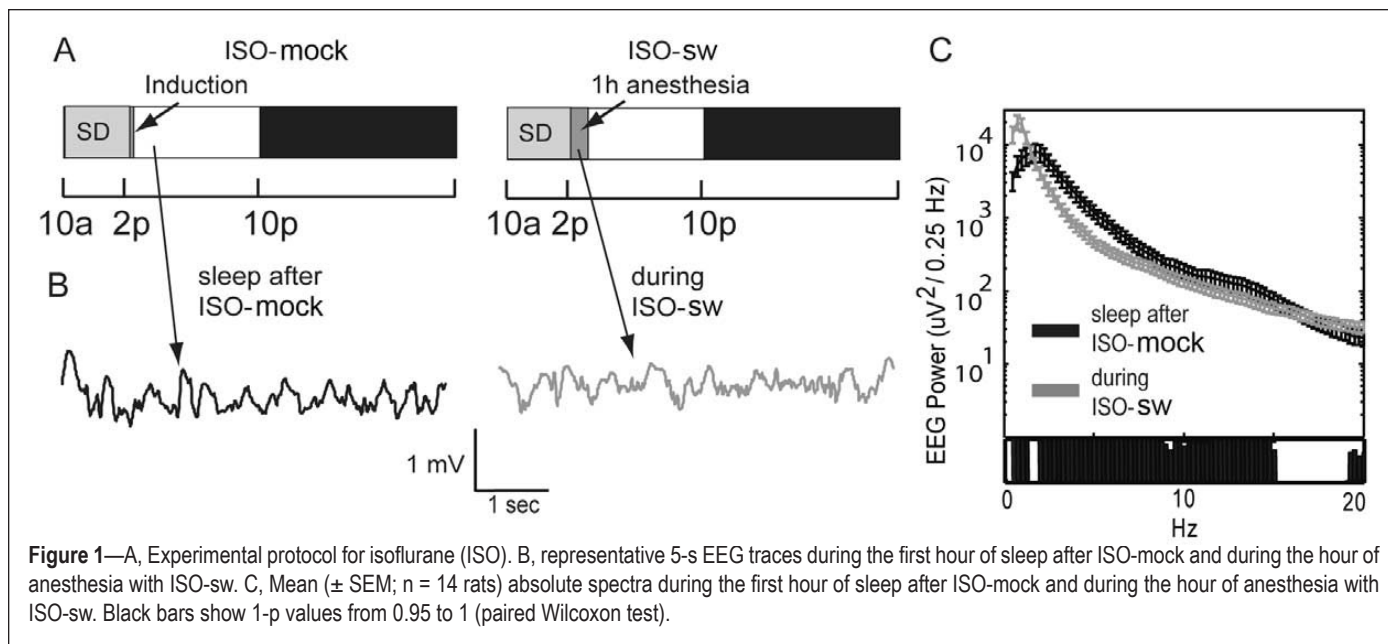
Rats were connected by means of a flexible cable and a commutator to a Grass mod. 8 polygraph (Grass Instruments, West Warwick, RI). EEG and EMG signals were conditioned by analog filters (EEG = high pass, 0.1 Hz; low pass, 35 Hz; EMG = high pass, 5 Hz; low pass, 70 Hz), digitized at 128 Hz (Kissei America, Irvine, CA), and stored on a computer. Wake, NREM sleep, and REM sleep were manually scored off-line (Sleep Sign; Kissei COMTEC, Matsumoto, Japan) in 4-s epochs according to standard criteria. Artifacts were always removed simultaneously from all derivations, and vigilance states could always be determined. EEG power spectra (fast Fourier transform routine, Hanning window) were calculated for consecutive 4-s epochs within the 0.25–20.0 Hz frequency range. In each rat a frontal and parietal channel showing a stable signal across all conditions were used for final analysis. Since results were similar in the 2 derivations, only data from the frontal channels are presented. SWA was computed for each epoch as the mean power from 0.5 to 4 Hz. SWE for each 24-h period was computed as the cumulative sum of SWA over time (equivalent to computing the product of NREM duration and 24-h mean SWA).

### Sleep Deprivation

Total sleep deprivation began at light onset and was performed for 4 hours by exposing the animals to a variety of novel objects and by transferring bedding material between cages. If rats became inactive and began to exhibit slow waves visible via real-time EEG monitoring, a new object was inserted into the cage, the cage was tapped, or the rat was gently prodded. Rats were never disturbed when they were spontaneously awake and active.

### Anesthesia

Anesthesia was delivered via a mobile anesthesia apparatus (Vaporstick; Surgivet, Waukesha, WI) fitted with the appropriate vaporizer (ISO: tech 4; DES: tech 6, Ohmeda, Madison, WI). In all cases anesthesia was induced in a chamber placed



inside the home cage. Following loss of righting reflex and the appearance of EEG slow waves, rats were removed from the chamber and transferred to an anesthesia mask and placed on a heat blanket. Cortical temperature was measured in 2 animals receiving DES using chronically implanted thermistors. In both rats the decrease in temperature during anesthesia (both sw and iso) relative to wake was  $\sim 1^{\circ}\text{C}$ , similar or smaller than the decrease during NREM sleep relative to wake in the mock condition, suggesting that the use of the heat blanket prevented changes in brain temperature in our experimental conditions. A pulse oximeter (V3402; Surgivet, Waukesha, WI) was used to monitor heart rate and  $\text{O}_2$  saturation from a clamp on the hind foot.  $\text{O}_2$  saturation remained  $> 95\%$  throughout the duration of anesthesia in all experimental groups. Heart rate during both ISO-sw and DES-sw was close to published values during sleep<sup>23</sup> at the beginning of the experiment ( $\sim 330$  beats/min), and then gradually and slightly decreased during the hour of anesthesia (see Figure S2, supplementary material is available online only at [www.journalsleep.org](http://www.journalsleep.org)), while during DES-iso it remained steady across the hour ( $334 \pm 4.3$ ).

The burst suppression ratio (BSR), i.e., the % of time spent in isoelectric EEG (suppression), was calculated for each 4-s epoch. For ISO, the amplitude threshold to define suppression was conservatively set at  $\pm 15 \mu\text{V}$  as in,<sup>24</sup> and the minimum duration for an isoelectric event was set to 0.5 sec as in.<sup>25</sup> For DES, the threshold was determined for each rat individually as  $\pm 3$  standard deviations of the mean voltage of clearly non-bursting EEG activity (mean  $23.6326 \pm 1.8 \mu\text{V}$ ; method for threshold<sup>26</sup>). BSR calculation was verified with visual EEG scoring for each rat: epochs scored as “isoelectric EEG” had BSR close to 100%, while epochs dominated by slow waves had BSR close to 0%.

All rats were visually monitored during the recovery from anesthesia, and actively prevented from going to sleep until they exhibited coordinated and purposeful movements (exploration of the cage), which in all cases occurred within 10 min. Wake behavior (eating, drinking, grooming, exploring) and sleep posture post-anesthesia were also monitored via video, and the time of the first grooming period was recorded.

### Statistical Analysis

All statistics were performed using the MATLAB 2007, statistics toolbox. Since the normalized SWA and SWE were normally distributed on the experiment day, comparisons were done via paired  $t$ -test or, for multiple comparisons, 2-way ANOVA (Rat  $\times$  Condition) followed by post-hoc paired  $t$ -tests with Bonferroni correction. Behavioral measures and frequency bins (bin size 0.25 Hz, no correction for bin number) were assessed by a paired Wilcoxon test or, for multiple comparisons, a Friedman test followed by post-hoc paired Wilcoxon with Bonferroni correction.

## RESULTS

### Isoflurane Experiments

Each rat was used in 2 experiments, both preceded by an undisturbed baseline recording (Figure 1A). Each experiment started with 4 h of sleep deprivation (beginning at light onset), followed by either ISO-mock or ISO-sw in a counterbalanced design, with one week between each condition. In ISO-mock animals were immediately allowed to recover after anesthesia induction (2-3 min). In ISO-sw instead, anesthesia lasted for  $\sim 1$  hour, and the concentration of the anesthetic (0.5% to 1.5%) was closely checked to maintain an EEG tracing with slow waves as similar as possible to the slow waves of NREM sleep (Figure 1B). Indeed, the BSR during ISO-sw was very low (mean  $\pm$  SEM,  $3.5\% \pm 0.9\%$ ,  $n = 14$  rats), indicating that very few epochs of isoelectric EEG occurred throughout the hour of anesthesia (see Table S1 and Figure S1, supplementary material is available online only at [www.journalsleep.org](http://www.journalsleep.org)), and the EEG power spectrum during the hour of anesthesia was dominated by large slow waves, similar to those observed during the first hour of sleep after ISO-mock. However, during anesthesia there was more EEG power in the lowest frequencies of the spectrum, corresponding to the low range SWA (0.5-1.5 Hz), and less power in the 2-15 Hz range (Figure 1C).

All rats recovered coordinated purposeful movements soon after anesthesia, but the latency to the first grooming episode was  $\sim 11$  min longer after ISO-sw relative to ISO-mock (Table

**Table 1**—Sleep parameters after isoflurane (A) and desflurane (B) anesthesia

	A		B		
	ISO-mock	ISO-sw	DES-mock	DES-sw	DES-iso
<b>Sleep Latency</b>	9.6 (2.0)	12.9 (2.1)	14.9 (4.8)	16.5 (3.0)	11.2 (4.0)
<b>REM Latency</b>	46.4 (3.1)	40.2 (2.9)	46.6 (3.9)	42.6 (4.8)	40.3 (5.2)
<b>1st Grooming</b>	14.3 (2.4)	25.5 (3.0)*	7.9 (1.2)	13.0 (2.8)	25.0 (4.3)*
<b>NREM sleep (min)</b>	461.2 (7.2)	471.7 (7.6)	447.0 (9.9)	458.8 (11.5)	466.6 (9.5)
<b>REM sleep (min)</b>	122.7 (5.2)	128.2 (4.6)	126.9 (5.4)	129.4 (3.9)	130.2 (7.0)
<b>Wakefulness (min)</b>	529.9 (10.4)	514.0 (9.8)	547.5 (13.0)	533.1 (14.0)	528.2 (6.5)
<b>Brief awakenings</b>	31.2 (2.0)	31.3 (2.2)	27.9 (0.9)	29.3 (2.0)	26.1 (1.5)

Mean sleep values ( $\pm$  SEM in parenthesis) refer to the period (~15:00-22:00) from the onset of consolidated sleep until the end of the dark period. The number of brief awakenings (< 16 sec) is expressed per hour of sleep. Paired Wilcoxon test, \* $P < 0.05$  (mock vs low).

1A). On the other hand, latency to sleep, sleep amount, and other sleep parameters calculated from sleep onset to the beginning of the dark phase (from ~15:00 to 22:00) did not differ between the 2 experimental conditions (Table 1A). Thus, one hour of anesthesia did not seem to affect either sleep duration or the overall sleep pattern.

After ISO-mock the relative EEG power spectrum during the first 4 h of NREM sleep was increased relative to baseline over most of the SWA range (0.5-3 Hz, Figure 2A), consistent with the changes observed in sleep deprived rats.<sup>18</sup> SWA as a whole (0.5-4.0 Hz) was also significantly increased in ISO-mock compared to baseline for the first 4 h after sleep onset ( $+27.2\% \pm 6.3\%$ ,  $P = 0.022$ , paired  $t$ -test), and a negative rebound was observed afterwards (Figure 2B), again consistent with the SWA response after sleep deprivation.<sup>27</sup> In a separate group of 9 rats that underwent 4 h of sleep deprivation starting at light onset (without any anesthesia) the SWA increase during the first 4 h of recovery sleep was  $+30.5\% \pm 3.8\%$  ( $P = 0.027$ , paired  $t$ -test), very similar to that observed after ISO-mock, strongly suggesting that anesthesia induction per se does not affect the SWA rebound. Analysis of the cumulative changes in SWA (SWE) during ISO-mock showed that the SWA lost during the 4 h of sleep deprivation was fully recovered before the end of the light period, with no changes during the following dark period (Figure 2C).

After ISO-sw the EEG power spectrum during the first 4 h of NREM sleep was also increased relative to baseline in the low range SWA (Figure 2D). In contrast to ISO-mock however, there was a decrease in the higher frequencies that spanned from 2.75 to 8 Hz, including the upper end of SWA (Figure 2D). SWA as a whole (0.5-4.0 Hz) was increased only at sleep onset; across the first 4 h of sleep there was a nonsignificant increase ( $+8.6 \pm 7.7$ ,  $P = 0.276$ , paired  $t$ -test) compared to baseline (Figure 2E). Moreover, the SWA increase from baseline was significantly smaller than the increase observed after ISO-mock ( $P = 0.0006$ , paired  $t$ -test). As for ISO-mock, SWA showed a negative rebound starting 4 hours after sleep onset. By the end of the dark period SWE after ISO-sw was still lower (86.6%) than in baseline (Figure 2F, light grey line), and lower than after ISO-mock ( $P = 0.018$ , paired  $t$ -test). When SWE was calculated including the SWA that occurred during the hour of anesthesia this was no longer the case: the SWA lost during sleep deprivation was quickly “recovered” within a few hours after the end of anesthe-

sia (Figure 2F, dark grey line), and SWE after ISO-sw and after ISO-mock no longer differed from each other.

We compared the relative spectra after ISO-sw and after ISO-mock during the first 3 hours after the end of anesthesia. During NREM sleep we found no differences in SWA but a decrease after ISO-sw in the 10-16 Hz frequency range, which encompasses sleep spindles (Figure 2G). Furthermore, the relative spectra during REM sleep differed around 6 Hz and above 16 Hz (Figure 2H), but were very similar during wake (Figure 2I).

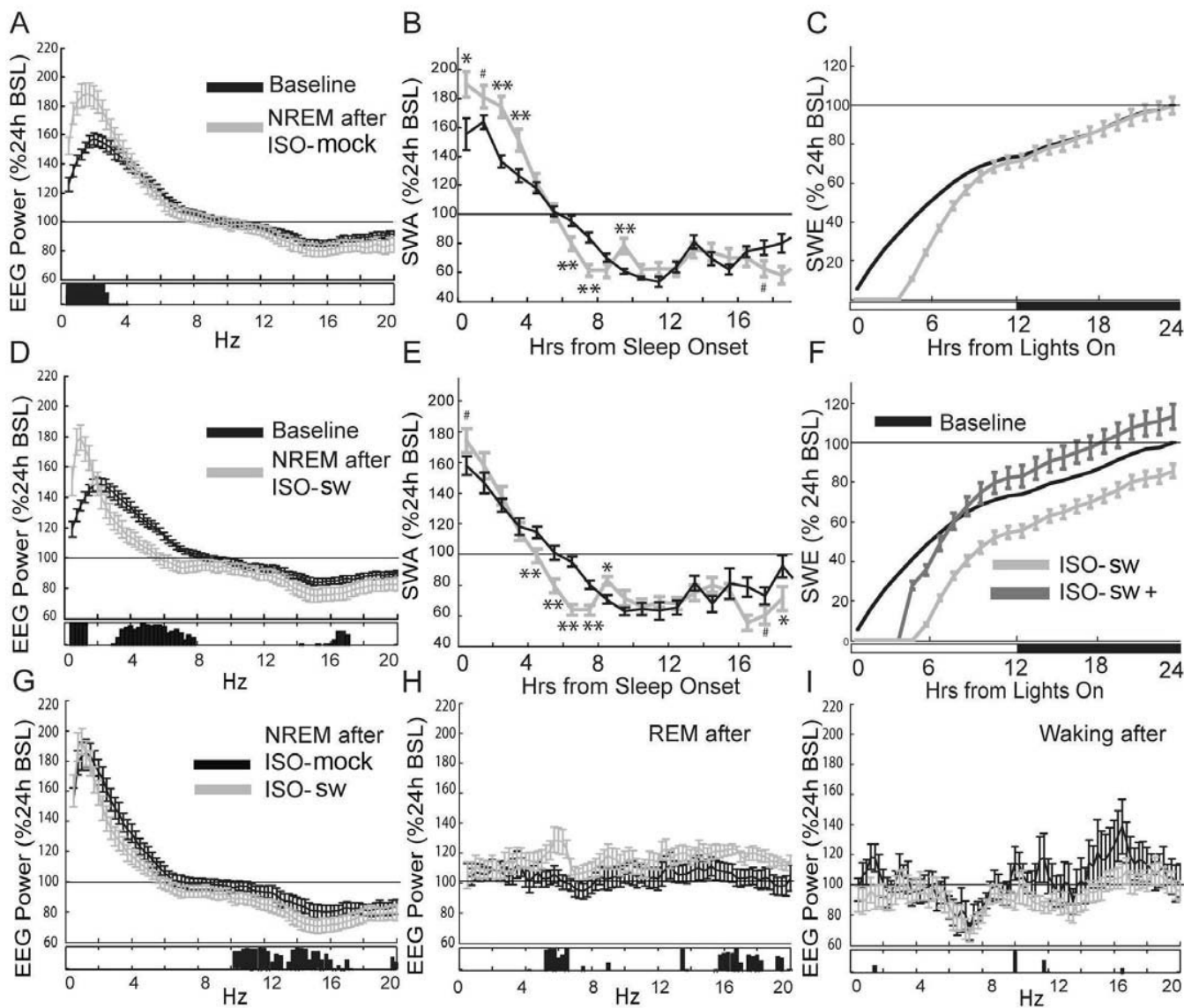
### Desflurane Experiments

Each rat was used in 3 experiments, all preceded by an undisturbed baseline recording (Figure 3A). DES-mock and DES-sw replicated the corresponding ISO conditions, except that a higher concentration of DES (4%-12%) was used. For DES-iso rats were exposed to DES for 1 hour at a constant concentration of 12%. As expected, DES-sw had low BSR (mean  $\pm$  SEM,  $5.8\% \pm 1.1\%$ ,  $n = 8$  rats; see also Table S1 and Figure S1), and the EEG power spectrum during DES-sw was dominated by large amplitude slow waves (Figure 3B). DES-iso, on the other hand, was characterized by a predominantly flat EEG signal (mean BSR  $90.9\% \pm 0.7\%$ ,  $n = 8$  rats; Table S1 and Figure S1), rarely interrupted by burst firing (Figure 3B). Similar to what was observed with ISO, the absolute EEG power during DES-sw was dominated by very low frequencies (0.5-1.5 Hz), while the power in most other frequencies (1.75-18 Hz) was decreased relative to the first hours of sleep after DES-mock (Figure 3C).

DES has a much lower solubility in fat than ISO, and thus leaves the tissue more quickly.<sup>28</sup> Consistent with a faster recovery time, the first grooming episode occurred 13 min earlier after DES-sw than after ISO-sw ( $P = 0.02$ , Wilcoxon), while the timing was similar for DES-iso and ISO-sw ( $P = 0.6$ , Wilcoxon; Table 1B). Sleep architecture was largely unchanged after DES-sw and DES-iso relative to DES-mock, as detailed in Table 1B.

After DES-mock the relative EEG power spectrum during the first 4 hours of NREM sleep was increased relative to baseline for most frequency bins, with the most prominent increase occurring in a cluster that spans SWA (Figure 4A). Relative SWA indeed increased compared to baseline during the first 5 h after sleep onset (Figure 4B), and as with ISO-mock the SWA of the first 4 h after sleep onset was higher than baseline ( $+37.08 \pm 3.9$ ,  $P < 0.001$ , paired  $t$ -test). The analysis of SWE showed that the SWA lost during the 4 hours of sleep deprivation was fully recovered before the end of the light period, with no further changes during the following dark period (Figure 4C).

After DES-sw the relative EEG power spectrum during the first 4 hours of NREM sleep showed a modest increase within the low-range SWA band (0.5-1.75) and an increase above 8 Hz (Figure 4D). SWA as a whole (0.5-4.0 Hz) was significantly increased during the 3rd, 4th, and 11th hours after sleep onset, and decreased during hours 6-8 (Figure 4E). Unlike after ISO-sw, SWA after DES-sw was higher compared to baseline during the first 4 hours after sleep onset ( $+14.04 \pm 4.7$ ,  $P = 0.042$ , paired  $t$ -test). Still, the SWA increase was significantly lower

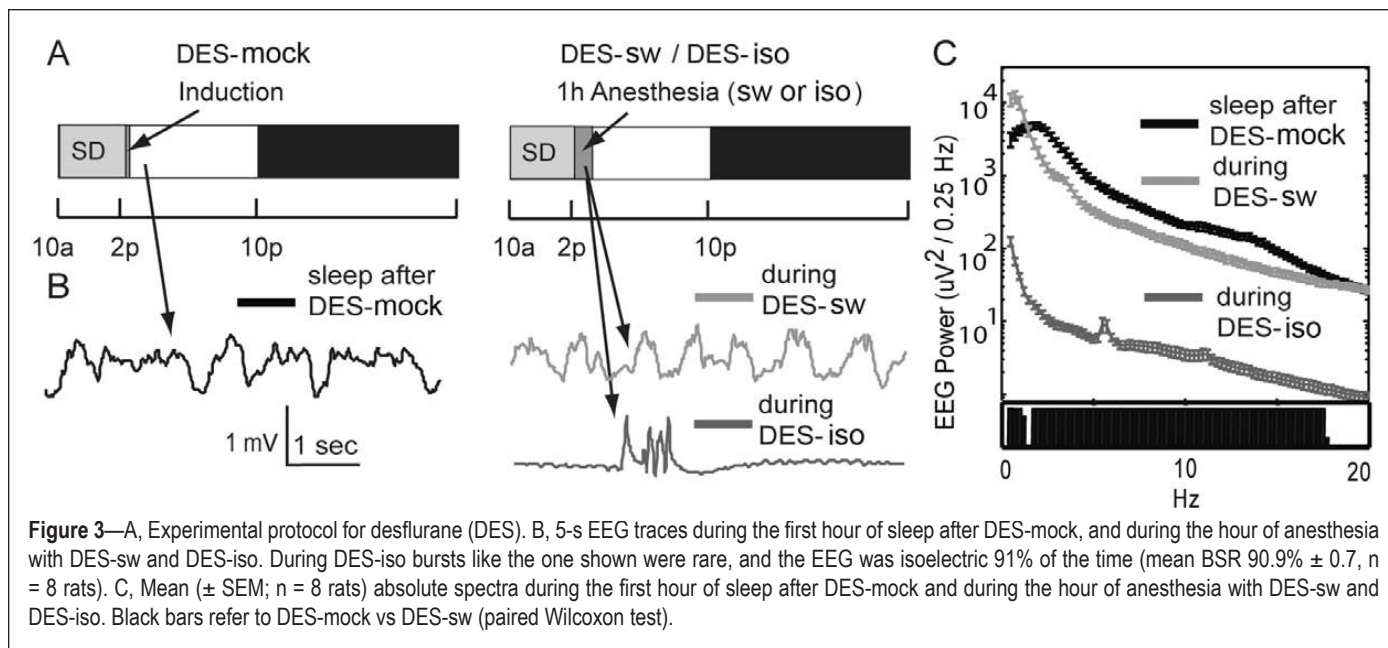


**Figure 2**—Effects of ISO anesthesia on SWA and SWE. (A–C) ISO-mock, (D–F) ISO-sw. Values are means ( $\pm$  SEM;  $n = 14$  rats). A and D, relative EEG power spectrum in NREM sleep during the first 4 h (expressed as % of the 24-h baseline). B and E, time course of relative SWA in NREM sleep on experiment day and corresponding baseline. C and F, cumulative SWE on experiment day and corresponding baseline. ISO-sw+ includes the SWE during the hour of anesthesia. (G–I) Relative EEG power spectra for 3 h (15:00–18:00) during NREM sleep (G), REM sleep (H), and Wake (I) for ISO-mock and ISO-sw. Black bars show 1- $p$  values from 0.95 to 1 (paired Wilcoxon test). #  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$  (paired  $t$ -test).

after DES-sw as compared to DES-mock ( $P = 0.0399$ , paired  $t$ -test). By the end of the dark period SWE after DES-sw was still lower (91%) than in baseline (Figure 4F, light grey line). As was the case for ISO however, when the SWA that occurred during anesthesia was included SWE surpassed baseline levels a few hours after the end of anesthesia (Figure 4F, dark grey line). By the end of the day SWE after DES-mock and after DES-sw no longer differed from each other.

After DES-iso the relative EEG spectrum during the first 4 hours of NREM sleep showed a modest increase within the low-range SWA band (0.5–2 Hz) and an increase in the 4.5–13 Hz range (Figure 4G). SWA as a whole (0.5–4.0 Hz) increased during the first 3 hours of sleep, and decreased after the 4th hour (Figure 4H); when calculated across the first 4 hours after sleep onset, SWA after DES-iso increased compared to baseline ( $+15.67 \pm 3.3$ ,  $P = 0.044$ , paired  $t$ -test), but this SWA rebound

was significantly lower than after DES-mock ( $P = 0.0465$ , paired  $t$ -test) and not different from DES-sw. SWE analysis showed that the SWA lost during the 4 hours of sleep deprivation was not recovered by the end of the following dark period (Figure 4I), when SWE was still 86% of baseline. SWE calculated separately for each of the following 2 recovery days remained lower than baseline (Figure 4I, inset). SWE after DES-iso also remained significantly lower than after DES-mock on both the first and second recovery day (Rec 1:  $P = 0.0391$ , Rec 2:  $P = 0.0313$ , paired-Wilcoxon), while SWE after DES-sw did not differ from SWE after DES-mock on either day. Finally, we compared the relative spectra after all 3 conditions during the first 3 hours after the end of anesthesia. Relative EEG spectra in all behavioral states were similar, except for a difference between DES-iso and DES-sw above 16 Hz in REM sleep (Figure 4J–L).



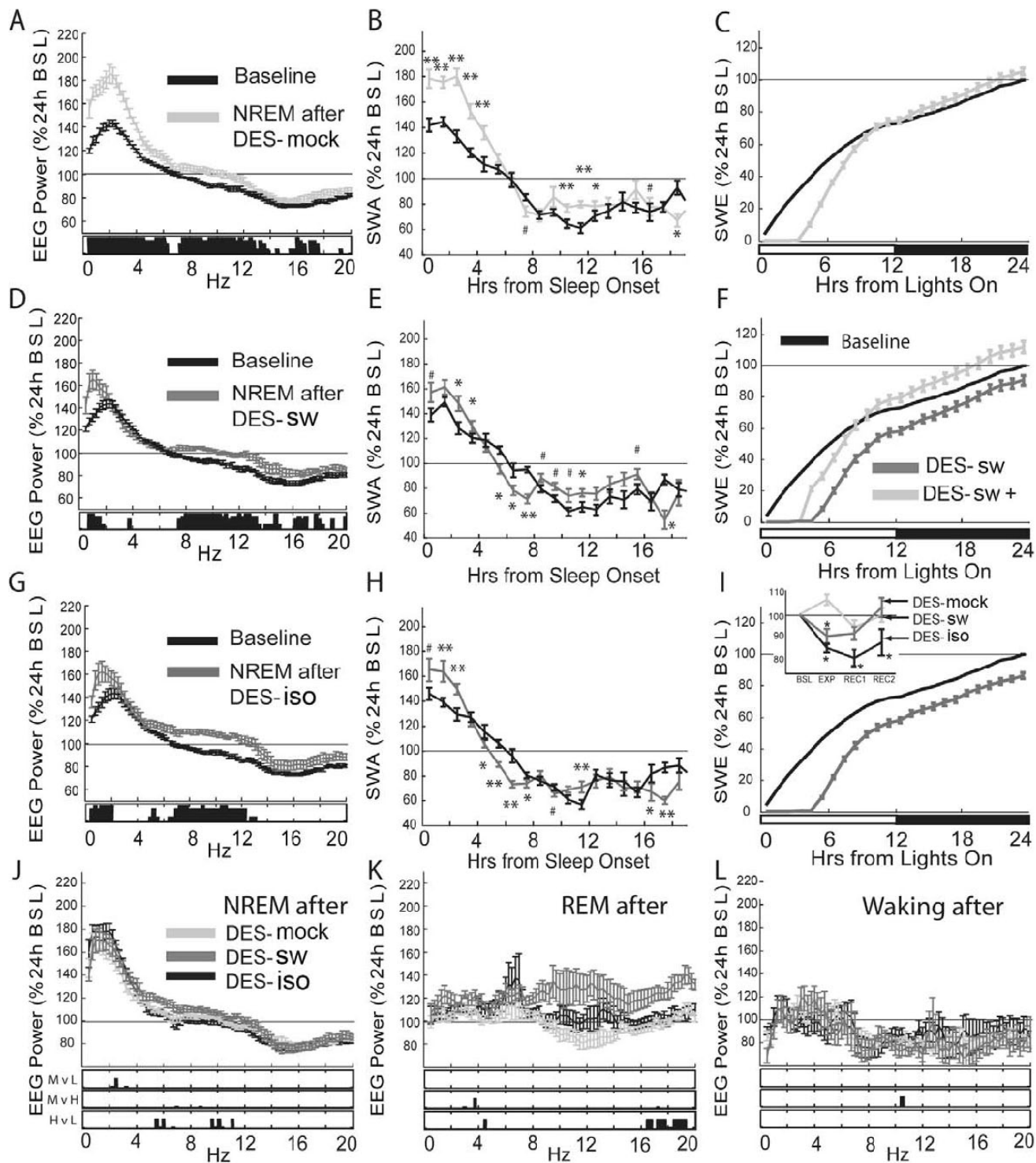
## DISCUSSION

SWA is an established marker of sleep pressure that is high at sleep onset and low after sleep. A seminal study demonstrated that the occurrence of slow waves during NREM sleep is necessary to reduce sleep need.<sup>13</sup> Specifically, a deprivation of slow waves during the first part of the night resulted in a subsequent increase in SWA, demonstrating that the occurrence of slow waves, not simply the time spent asleep, decreases SWA. Thus, we reasoned that if anesthesia slow waves count as sleep slow waves, then anesthesia slow waves should cause a homeostatic decrease in SWA. In agreement with this hypothesis we found that one hour of ISO or DES anesthesia associated with the occurrence of large, almost continuous slow waves reduced the SWA rebound that was expected following 4 hours of sleep deprivation. Moreover, when anesthesia slow waves were included in calculating cumulative SWA (SWE), the latter returned to baseline levels within a few hours after the end of anesthesia. Thus, anesthesia slow waves may substitute for sleep slow waves.

These findings are consistent with previous studies in rats that showed that 12 hours of propofol anesthesia during the normal sleep period do not increase subsequent sleep pressure,<sup>29</sup> and that 6 hours of either propofol anesthesia or undisturbed sleep allow for a similar recovery from 24 hours of sleep deprivation.<sup>17</sup> Our ISO-sw and DES-sw conditions are likely comparable to the propofol condition in these reports (described as producing continuous high-amplitude tracing), and propofol, ISO and DES have similar mechanisms of action, in that they are primarily GABA(A) agonists.<sup>30</sup> In contrast to the propofol studies, however, we could measure the effects of anesthesia on the lowest EEG frequencies, and found that the EEG power spectrum during ISO-sw and DES-sw was skewed towards frequencies below 1.5 Hz, which correspond to the slow oscillation. The link between slow oscillation and SWA is complex. The former is the cellular phenomenon that underlies the occurrence of NREM slow waves, and thus SWA. Yet, several reports<sup>31–34</sup> have suggested a possible dissociation between the homeostatic behavior of frequencies below 1 Hz and the remaining SWA frequencies. More recent studies in rodents

and humans, however, as well as computer simulations, show that the apparent dissociation may simply reflect homeostatic changes in the slope of slow waves.<sup>35,36</sup> Specifically, the decrease of homeostatic sleep pressure in the course of sleep is associated not only with an overall decrease in SWA, but also with a redistribution of the EEG power spectrum towards lower frequencies, due to a decrease in the slope of slow waves.<sup>35</sup> In this respect, the EEG during anesthesia is more similar to the EEG during late sleep, when sleep pressure is low, than to the EEG during early sleep, when sleep pressure peaks. This observation is consistent with the notion that anesthesia can in itself discharge previous sleep pressure due to sleep deprivation.

One hour of anesthesia with high concentration of DES, associated with isoelectric EEG and few slow waves, also reduced the SWA rebound following 4 hours of sleep deprivation. This finding was unexpected, since the results with ISO-sw and DES-sw, as well as a previous study,<sup>13</sup> were consistent with the idea that slow waves are necessary to discharge sleep pressure. One possible explanation is that the blunted “SWA rebound” after DES-iso reflects not a physiological decrease in sleep need, but rather a pathological condition that may last long after the end of anesthesia. Consistent with this interpretation, the SWA lost during sleep deprivation and DES-iso was not recovered even 2 days after the end of anesthesia, when SWE was still  $\sim 85\%$  of baseline. It is possible that the exposure to 12% DES in the DES-iso condition may have caused hypotension, acidemia, and possibly ischemia, and that had these effects been prevented, the chronic decrease in SWE would not have occurred. Of note, however, there were no obvious abnormalities in the EEG signal after DES-iso, and the chronic decrease in the EEG power spectrum was specific for SWA. Another possibility, therefore, is that what counts to discharge sleep pressure is not the presence of slow waves per se, but some other cellular phenomena that normally are associated with them. These phenomena may include the repeated alternation between up and down states, as during the slow oscillation, and/or the occurrence of relatively long periods of neuronal silence (as in the down states of the slow oscillation). Interestingly, recent in-



**Figure 4**—Effects of DES anesthesia on SWA and SWE. (A-C) DES-mock, (D-F) DES-sw, (G-I) DES-iso. Values are means ( $\pm$  SEM;  $n = 8$  rats). A, D, and G, relative EEG power spectrum in NREM sleep during the first 4 h (expressed as % of the 24-h baseline). B, E, and H, time course of relative SWA in NREM sleep on experiment day and corresponding baseline. C, F, and I, cumulative SWE on experiment day and corresponding baseline. DES-sw+ includes the SWE during the hour of anesthesia. Inset in I, 24-h SWE across days on baseline (BSL), experiment day (EXP), 1st recovery day (REC1), and 2nd recovery day (REC2). Due to a technical problem one rat could not be used to compute DES-iso SWE on the 2nd recovery day. (J-L) Relative EEG power spectra for 3 h (15:00-18:00) during NREM sleep (J), REM sleep (K), and Wake (L) for DES-mock, DES-sw, and DES-iso. Black bars show 1-p values for mock vs. sw (m v sw), mock vs. iso (m v iso), and iso vs. sw (iso v sw) (paired Wilcoxon test). #  $P < 0.1$  \*  $P < 0.05$ , \*\*  $P < 0.01$  (paired  $t$ -test or Wilcoxon).

tracellular recordings were performed in cats anesthetized with high doses of ISO, which produced a burst-suppression pattern (Figure 3B). As expected, it was found that during the suppression phase with isoelectric EEG neurons were chronically

hyperpolarized and silent. Surprisingly, however, it was also found that during the burst phases neurons were not tonically depolarized, but oscillated between up and down states as during normal sleep or during low doses of anesthesia.<sup>37</sup> Thus, the

DES-iso condition that we used in this study, in which bursts were present although rare, shares at least some of the features of the DES-sw condition. It remains to be explained, however, why the reduced sleep need presumably caused by DES-iso continues for at least 2 days.

Whether NREM sleep and anesthesia have similar cellular consequences is unclear. Few transcriptomic and proteomic studies have examined changes in brain gene expression after anesthesia, and direct comparisons with similar studies that compared sleep and wake states are difficult, due to major differences in experimental design and statistical analysis. On one hand, sleep seems to be associated with increased expression of genes related to synaptic depression, protein synthesis, cholesterol synthesis, and membrane trafficking.<sup>38</sup> On the other hand, one report found hardly any changes at the mRNA or protein level following clinically relevant and repeated doses of ISO,<sup>39,40</sup> while other studies found significant changes in gene expression long after the end of anesthesia,<sup>41,42</sup> and yet another study found changes in membrane proteins, but only 3–72 hours after anesthesia.<sup>43</sup>

The mechanisms of action of volatile anesthetics remain poorly understood, and most likely include pre- and postsynaptic effects at both subcortical and cortical level. The slow oscillation of NREM sleep occurs in most thalamocortical neurons, and intracellular recordings have shown that during the down state of this oscillation cortical neurons are disfacilitated, rather than inhibited; in other words, they stop firing due to the absence of excitatory synaptic activity, and not because of the activation of GABA(A) inhibitory currents.<sup>44</sup> Disfacilitation, and not direct inhibition, also occurs under anesthesia with ketamine-xylazine or urethane,<sup>45</sup> but unfortunately volatile anesthetics have not been studied. Interestingly, however, a recent study found that the cerebral cortex is a major target of volatile anesthetics, and that these agents decrease cortical firing by enhancing neocortical GABA(A) receptor-mediated inhibition.<sup>46</sup> Assuming that this is the predominant mechanism by which ISO and DES affect cortical activity, it would follow that ISO and DES slow waves and sleep slow waves are caused by different cellular mechanisms, involving inhibition for the former and disfacilitation for the latter.

Although its functions remain elusive, there is compelling evidence that sleep benefits the brain and improves vigilance, attention, learning, and memory. The underlying mechanisms are unclear, and may involve overall synaptic renormalization in most brain regions<sup>47</sup> and/or synaptic strengthening of restricted neural circuits.<sup>48</sup> Our results based on SWA analysis suggest that, as far as the homeostatic regulation of sleep is concerned, anesthesia may “count” as NREM sleep. One recent study in mice also suggested that anesthesia may counterbalance some of the negative effects of sleep loss on performance and synaptic plasticity. Specifically, it was found that a day after 2 hours of ISO anesthesia (presumably with slow waves) mice showed improved visuospatial learning and enhanced hippocampal LTP, and both effects were mediated by the increased hippocampal expression of the NR2B subunit of NMDA receptors.<sup>49</sup> Of note, sleep restriction and total sleep deprivation impair spatial learning and the induction of hippocampal LTP, and one of the underlying mechanisms seems to be a decrease in NMDA currents, associated with an increase in the NR2A/NR2B ratio.<sup>50–54</sup> These results, however, do not mean that anesthesia should be

considered as an option to counteract the effects of sleep deprivation, since most available evidence suggests that it cannot provide the restorative functions of sleep. For instance, a study in rats found that 2 hours of anesthesia (ISO / NO mixture) impairs the acquisition of a spatial memory task for several weeks.<sup>55</sup> Most importantly, postoperative cognitive dysfunction is a well described decline in cognitive performance observed at all ages after a surgery performed under anesthesia,<sup>56,57</sup> and growing evidence suggests that anesthesia may contribute to the development of Alzheimer disease.<sup>58</sup>

In summary, we find that two different doses of volatile anesthetics can blunt the SWA response to sleep deprivation. Slow waves were present only with the lower doses, suggesting that their presence may not be necessary to decrease the sleep pressure accumulated during sleep deprivation. The repeated occurrence of slow oscillations in corticothalamic neurons, and/or multiple, sustained periods of hyperpolarization and neuronal silence in these cells may be required to decrease sleep pressure. Unfortunately, these two possibilities are difficult to distinguish, because in our experience it is not possible to maintain isoelectric EEG without any burst activity for an entire hour.

This study has several limitations. We cannot rule out that anesthesia, especially at high doses, affected cardiovascular and respiratory functions. However, heart rate and oxygen saturation were monitored and remained within normal levels for all rats. We used only one hour of anesthesia, following 4 hours of sleep deprivation in order to produce a reliable sleep rebound and a detectable anesthetic effect, while still allowing recovery during the light period, when rats normally sleep. Longer periods of anesthesia may produce different results. Finally, sleep pressure was measured using SWA; functional studies that examine the recovery of attention and performance after sleep loss followed by anesthesia should also be performed.

## ACKNOWLEDGMENTS

The work was supported by NIMH award 1P20MH077967 to Chiara Cirelli, the NIH Director’s Pioneer award to Giulio Tononi, and NIH Training grant T32-GM007507 to Aaron B. Nelson. We thank Danielle Reis for technical assistance and Dr. Vladyslav V. Vyazovskiy for helpful discussions.

## DISCLOSURE STATEMENT

This was not an industry supported study. Dr. Tononi has received research support from Respiroics and participated in speaking engagements for Sanofi-Aventis and Respiroics.

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