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Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness

J.T. McKenna¹, J.L. Tartar^{1,2}, C.P. Ward¹, M.M. Thakkar¹, J.W. Cordeira¹, R.W. McCarley^{1,2}, and R.E. Strecker¹

¹ Boston VA Healthcare System and Harvard Medical School, Laboratory of Neuroscience, VAMC Research 151-C, 940 Belmont Street, Brockton, MA 02301.

² Boston VA Healthcare System and Harvard Medical School, Laboratory of Neuroscience, in vitro electrophysiology group, VAMC Research 151-C, 940 Belmont Street, Brockton, MA 02301.

Abstract

Sleep fragmentation, a feature of sleep apnea as well as other sleep and medical/psychiatric disorders, is thought to lead to excessive daytime sleepiness. A rodent model of sleep fragmentation was developed (termed **sleep interruption, SI**), where rats were awakened every 2 min by the movement of an automated treadmill for either 6 or 24 h of exposure. The sleep pattern of rats exposed to 24h of SI resembled sleep of the apneic patient in the following ways: sleep was fragmented (up to 30 awakenings/h), total REM sleep time was greatly reduced, NREM sleep episode duration was reduced (from 2 min, 5 s baseline to 58 s during SI), whereas the total amount of NREM sleep time per 24h approached basal levels. Both 6 and 24 h of SI made rats more sleepy, as indicated by a reduced latency to fall asleep upon SI termination. Electrographic measures in the recovery sleep period following either 6 or 24 h of SI also indicated an elevation of homeostatic sleep drive; specifically, the average NREM episode duration increased (e.g., for 24 h SI, from 2 min, 5 s baseline to 3 min, 19 s following SI), as did the NREM delta power during recovery sleep. **Basal forebrain (BF)** levels of extracellular **adenosine (AD)** were also measured with microdialysis sample collection and HPLC detection, as previous work suggests that increasing concentrations of BF AD are related to sleepiness. BF AD levels were significantly elevated during SI, peaking at 220% of baseline during 30 h of SI exposure. These combined findings imply an elevation of the homeostatic sleep drive following either 6 or 24 h of SI, and BF AD levels appear to correlate more with sleepiness than with the cumulative amount of prior wakefulness, since total NREM sleep time declined only slightly. SI may be partially responsible for the symptom of daytime sleepiness observed in a number of clinical disorders, and this may be mediated by mechanisms involving BF AD.

Corresponding Author: James T. McKenna, PhD, Harvard Medical School and Boston VA Healthcare System, VAMC Research 151-C, 940 Belmont Street, Bldg. 46, Brockton, MA 02301. Phone: 508-583-4500 x61879, Fax: 508-895-0153, E-mail: james_mckenna@hms.harvard.edu.

Current addresses: JLT: Nova Southeastern University, Farquhar College of Arts and Sciences, Division of Social and Behavioral Sciences, 3301 College Avenue, Fort Lauderdale, FL 33314.

CPW: University of Florida, Department of Physiological Sciences, PO Box 100144, Gainesville, FL 32610.

MMT: Harry Truman VA Medical Hospital and University of Missouri, Department of Neurology, One Hospital Drive, Columbia, MO 65212.

JWC: Tufts University School of Medicine, Department of Neuroscience, 136 Harrison Avenue, Boston, MA 02111.

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Keywords

adenosine; sleep; EEG (electroencephalogram); rat; microdialysis; forebrain; homeostasis; apnea; delta; sleep latency

Obstructive sleep apnea (OSA) affects 2–4% of the general population (Young et al., 1993), and is characterized by apneic moments during sleep, which are terminated by brief arousals and a reestablishment of the upper airway patency. Thus, sleep fragmentation is a primary physiological disturbance, and may be responsible for some of the symptoms/signs associated with OSA, including excessive daytime sleepiness, which is a principal presenting complaint of patients (Roth et al., 1995; Roth et al., 1995; Roth and Roehrs, 1996; Day et al., 1999). Furthermore, patients suffering from a variety of psychiatric, medical, and sleep/respiratory disorders may also experience sleep fragmentation leading to excessive daytime somnolence and impairments of both attention and memory (for review, see Hoyt, 1995; Kryger et al., 2000; Sateia et al., 2000; Soldatos and Paparrigopoulos, 2005). Interestingly, the effects of sleep fragmentation may be due to the periodic disturbances, since total sleep time may only diminish slightly (Coleman et al., 1982; Bonnet, 1987; Stepanski, 2002). It has been proposed that the sleep fragmentation experienced by OSA patients may particularly lead to daytime hypersomnolence. Although numerous studies have investigated the effects of total sleep deprivation or selective REM sleep deprivation on physiology and behavior, few have investigated the effects of sleep fragmentation, which may better model the sleep disturbances experienced in some human disorders.

The inhibitory neuromodulator **adenosine (AD)** has been proposed to be an endogenous sleep factor (for review, Radulovacki, 1985; Basheer et al., 2004). For example, systemic and intracerebral injections of AD have been shown to increase sleep, whereas AD antagonists, such as caffeine, increase arousal (Dunwiddie and Worth, 1982; Ticho and Radulovacki, 1991; Benington and Heller, 1995; Benington et al., 1995; Portas et al., 1997; Fulga and Stone, 1998; Fredholm et al., 1999; Mendelson, 2000). Recent work supports the hypothesis that AD facilitates sleepiness by inhibiting arousal-related neurons in the ventral **basal forebrain (BF)** region, which contains numerous cholinergic neurons (see Figure 5; Porkka-Heiskanen et al., 1997, 2000; Alam et al., 1999; Szymusiak et al., 2000; Strecker et al., 2000; Thakkar et al., 2003a,b; Basheer et al., 2004; Steriade and McCarley, 2005), although the role of BF AD as a putative regulator of the homeostatic sleep drive has been recently disputed (Blanco-Centurion et al., 2006). Extracellular AD levels have been found to rise selectively in the BF and cortex during sleep deprivation followed by a slow decline to baseline during recovery sleep, mirroring changes of the homeostatic sleep drive (Porkka-Heiskanen et al., 1997, 2000; Kalinchuk et al., 2003; McKenna et al., 2003; Murillo-Rodriguez et al., 2004).

The present study tested the hypotheses that, 1. Experimental sleep fragmentation (**sleep interruption, SI**) for 6 and 24 h increases sleepiness, and, 2. SI elevates BF AD levels. Sleepiness was assessed in the recovery period following SI using measures that include the latency to sleep onset (rodent multiple sleep latency test), average delta power in NREM sleep, and average NREM episode duration. AD levels in BF were determined by microdialysis sampling coupled to chromatographic analysis.

METHODS

Adult male Sprague-Dawley rats (Charles River Laboratories), weighing between 280 and 350 gms, were housed under constant temperature ($23 \pm 1^\circ\text{C}$) and 12:12 light:dark cycle (light-on period from 07:00 h to 19:00 h unless otherwise noted) with food and water available ad libitum.

All animals were treated in accordance with AAALAC's policy on care and use of laboratory animals.

Polysomnography methods

Electroencephalograph (EEG) and electromyograph (EMG) surgery was carried out under general anesthesia (sodium pentobarbital, 65 mg/kg, i.p.). Bilateral screw electrodes (Plastics One Inc. Roanoke, VA) were fixed onto the skull above the temporal cortex (2 mm caudal to bregma and 4 mm lateral to the mid-sagittal suture) for recording EEG. EMG electrodes, which consisted of flexible stainless steel wires insulated with nylon, except for a suture pad (1.62 mm) ending (Plastics One Inc.), were placed in the superior nuchal muscles. A Grass Model 15 polygraph with model 15A4 amplifiers (Grass-Telefactor, West Warwick, RI) was used for all EEG and EMG data collection. Behavior was classified into 3 different states by means of EEG and EMG analysis: wakefulness (W) – low-amplitude, high frequency desynchronized EEG accompanied by active muscle tone evident in EMG; nonREM sleep (NREM, also referred to as slow wave sleep) – low-frequency, high-amplitude synchronized EEG accompanied by low muscle tone; and rapid eye movement sleep (REM) – desynchronized EEG accompanied by absence of muscle tone. Grass Rodent Sleep Stager (RSS) V3.0 (Grass-Telefactor) was used for off-line EEG and EMG analysis. Recordings were visually scored in 10 s epochs as previously described (Thakkar et al., 2003b). Increased delta power (1–4 Hz) in NREM during recovery sleep following sleep deprivation has been shown to be an indicator of the homeostatic regulation of sleep (Borbely and Neuhaus, 1979; Borbely, 1982; Franken et al., 1991). Spectral analysis of the EEG, employing Fast Fourier Transform, was performed to investigate delta power in NREM sleep (that is, NREM delta power per hour of NREM) during recovery. In particular, EEG delta power was measured per 10 s epoch by the Grass Gamma EEG acquisition system V4.6 (Grass-Telefactor), where the delta frequency range was set at 1–4 Hz, in order to avoid low frequency artifact. Spectral power was analyzed for all vigilance states (wake, NREM, and REM) for all 10 s epochs manually verified to be artifact-free. Vigilance state amounts and delta power measurements during SI and the recovery period after SI were compared to baseline day amounts in the same rats; i.e., each animal was used as its own control. NREM delta power was represented as average power per hour. That is, the absolute total NREM delta power amount in one hour was then divided by the amount of NREM time in that hour.

Microdialysis methods

Microdialysis experiments were undertaken in a different group of animals than those used in the polysomnographic studies. Under general anesthesia [i.p. sodium pentobarbital and an i.m.-injected combination of ketamine, xylazine and acepromazine], intracerebral guide cannulas were implanted. At least 16 h prior to the beginning of sample collection, a microdialysis probe (CMA11, 2 mm membrane length, 0.24 mm diameter; CMA/Microdialysis, Stockholm) was inserted through the implanted guide cannula into the BF (AP –0.3, L 1.8, H –9.0), or a non-BF rostral control region, including the ventral pallidum, nucleus accumbens, and caudate putamen (AP 0.2, L 2.0, H –9.0). Probe inlet and outlet tubing (FEP tubing; CMA/Microdialysis) transported artificial cerebrospinal fluid (aCSF, Harvard Apparatus, Holliston, Massachusetts) at a flow rate of 1.5 μ l/min. Samples were collected from the outlet tubing after exiting the test cage. For all experiments the time delay due to the dead volume of the system (fluid contained in the probe and outlet tubing) was taken into account in correlating neurochemical readings with lights on or lights off. As previously described (Porkka-Heiskanen et al., 1997, 2000; McKenna et al., 2003), 10 μ l microdialysis samples were analyzed with a microbore high performance liquid chromatography (HPLC) system coupled to a Waters 2487 UV detector (detection wavelength = 258 nm). For missing samples and for outliers (values that varied by > 2 S.D. compared to other samples from the same probe), values were assigned using an arithmetic mean of the surrounding data points (<1.5% of all samples).

Sleep interruption apparatus

The SI parameters were designed to model the frequency of sleep fragmentation observed in obstructive sleep apnea. Rats lived in a modified treadmill cage ($1 \times w \times h = 50.8 \text{ cm} \times 16.51 \text{ cm} \times 30.48 \text{ cm}$) in which the floor was a horizontal belt that was automatically programmed to move slowly at a rate of .02 m/s, which was a speed we determined to reliably produce awakenings. The treadmill ran at this slow speed for 30 s (30 s on), followed by no treadmill activity for 90 s (90 s off), continuously producing up to 30 interruptions of sleep per hour. As an exercise control group (EC), different rats were exposed to treadmill movement for 10 min on, followed by 30 min without treadmill movement, producing comparable overall amounts of movement/exercise between the SI and EC groups.

SI polysomnographic recording

Rats were habituated to the treadmill cages and EEG cables for 5 days with light/dark cycle 07:00/19:00. On days 4 and 5, the rats were exposed to 1 h of treadmill motion (5 min on: 5 min off) for each of these two days preceding SI exposure, in order to habituate the rat to its new environment and the movement of the treadmill. On day 6, the baseline EEG/EMG was recorded for 24 h. For all polysomnographic measurements during SI exposures and recovery, each rat was compared to his own baseline. Three different SI treatments were used in the polysomnography recording in order to facilitate comparisons with the SI microdialysis findings:

A) 6 h SI, treadmill off at the beginning of the dark period—For exposure day 7, recording started in 6 rats for 6 h of SI (13:00–19:00), and concluded with 3 h of recovery recording (19:00–22:00, beginning of the dark period).

B) 6 h SI, treadmill off 3 h before the end of the light period—For exposure day 7, recording started in 6 rats for 6 h of SI (10:00–16:00), and concluded with 3 h of recovery recording (16:00–19:00, still light period).

C) 24 h SI—For exposure days 7–8, recording started for 6 rats for 24 h of SI (at 13:00 on day 7) followed by a 6 h recovery period (starting at 13:00 on day 8). In another group of rats, 24 h of EC was performed, followed by a 6 h recovery period, matching the time periods of 24 hr SI exposure. This 24 h SI polysomnography study was designed to allow comparison to the 30 h microdialysis study described below.

Rodent multiple sleep latency test following SI exposures

Sleepiness can be defined operationally as “the propensity to fall asleep”. Hence, to obtain direct data on the sleepiness of experimental rats, a unique rodent multiple sleep latency test (MSLT) was used that contained features of the clinical MSLT and of procedures used in published animal studies (Shiromani et al., 1991; Veasey et al., 2004; Blanco-Centurion et al., 2006). The rodent MSLT was performed in the recovery period following 6 h SI with treadmill off at the beginning of the dark period (N=8); 6 h SI with treadmill off 3 h before the end of the light period (N=8); and 24 h SI (N=8). In another group of rats, 24 h of EC was performed, matching the time periods of 24 hr SI exposure (N=8). The rodent MSLT included six separate sleep latency tests/trials conducted within the three hour recovery period. For each of these six tests/trials, the rat was initially kept awake for five minutes by means of gentle handling (primarily auditory and light tactile stimulation, without explicit handling). Rats were then left alone for 25 min while polysomnographic data was collected. Unlike the human MSLT, rats were allowed to sleep during these 25 min periods. This test/trial was then repeated five more times on 30 min intervals. An elevation of the homeostatic sleep drive following SI treatment was assessed comparing the average across rats of the six sleep latency trials (the time to fall

asleep within the undisturbed 25 min period); this average was compared to the rat's six sleep latency trials on a baseline control day. In another group of rats, 24 h of EC was performed, matching the time periods of 24 hr SI exposure. The rodent MSLT was also performed in these animals (N=8), and results were compared to baseline and 24 hr SI latency values. This rodent multiple sleep latency test can be considered a direct measure of sleepiness, whereas the other measures (NREM episode duration and NREM delta power during recovery sleep) can be considered indicators of sleepiness (Veasey et al., 2004).

Microdialysis sampling during SI exposure and recovery

Rats were habituated to the treadmill cage, and treadmill movement (one hour per day) for two days preceding SI exposure. Microdialysis sampling was also undertaken using three comparable SI treatments:

A) 6 h SI, treadmill off at the beginning of the dark period—4 different groups were analyzed: 1) 7 animals were exposed to SI, where the treadmill turned on for 30 s: off for 90 s; 2) 6 sleep deprived animals, where animals were kept awake by gentle handling from 13:00–19:00 (these data are modified from McKenna et al., 2003); 3) 7 animals were in the **exercise control (EC)** group, where the treadmill turned on for 10 min: off for 30 min; and 4) 4 rats were **cage controls (CC)**, living undisturbed in the treadmill cage. 1 h samples were collected from BF beginning at 12:00 to obtain basal AD levels (12:00–13:00), followed by treatment exposure (13:00–19:00, where 19:00 is the beginning of the dark period), and concluded with a 3 h undisturbed recovery period in the dark (19:00–22:00). AD levels were normalized as percent of baseline AD levels (12:00–13:00).

B) 6 h SI, treadmill off 3 h before the end of the light period—3 different groups were analyzed: 1) 7 animals were exposed to SI; 2) 7 animals were included in the EC group; and 3) 4 animals were included in the CC group. 1 h samples were collected from BF beginning at 09:00 to obtain basal AD levels (09:00–10:00) followed by 6 h of exposure (10:00–16:00), and concluded with a 3 h undisturbed recovery period during the light period (16:00–19:00). AD levels were normalized as percent of baseline AD levels (09:00–10:00).

C) 30 h SI—We analyzed AD level fluctuation in the BF during 30 h exposure to SI 3 different groups were analyzed: 1) 8 animals were exposed to SI; 2) 3 animals were in a rostral control group in which the microdialysis probes were placed outside of our target region in the cholinergic BF (the non-BF rostral control group); and 3) 7 animals were in an exercise control group (EC). 6 h samples were collected from BF beginning at 07:00 to obtain basal AD levels (07:00–13:00) followed by 6 h samples of the second half of the light period with the treadmill on (13:00–19:00), then 12 h samples of treadmill on in the dark period (19:00–07:00 the following day), and then finally the 12 h of treadmill on in the light period of the following day (07:00–19:00). AD levels were normalized as percent of baseline AD levels (09:00–13:00).

Summary of Methods

Table 1 provides a protocol overview, where rows represent the SI exposure/treatment groups, and columns represent types of exposure, light/dark schedule of the experiment, time of exposure, duration (length) of exposure, and time of the 3 or 6 h recovery period where we evaluated the homeostatic sleep drive by means of polysomnographic analysis. All of the exposures were evaluated by the same criteria of polysomnographic measures of average sleep latency in the rodent MSLT during the first 3 h of recovery; average NREM delta power per hour of NREM during recovery and average NREM episode duration during recovery; and BF AD levels during exposures.

Statistical Methods

All parameters were analyzed using parametric repeated measures ANOVA, in order to compare treatment groups. If the main effect in the ANOVA was found to be significant, and more than two groups were compared, a pair-wise comparison between groups was then made, using Bonferroni correction. If a significant treatment X time interaction in the ANOVA was observed, this was followed by pair-wise comparisons of the data (grouped into 3, 6 or 12 h bins if appropriate), again using Bonferroni correction. Statistical analysis utilized SPSS software (release 11.5), and differences were determined to be significant when $P < .05$.

RESULTS

Effects of SI on the distribution of sleep and wakefulness

6 h SI, recovery at the beginning of the dark period—The total percent of time spent awake was significantly increased in animals exposed to 6 h of SI (repeated measures ANOVA, SI from 13:00–19:00, lights out at 19:00; from 29.2% baseline to 65.0% SI; $F_{1,10}=33.442$, $P<.001$). In turn, NREM percentages were decreased (from 62.0% to 34.7%; $F_{1,10}=22.681$, $P=.001$), as were REM (8.8% to 0.2%; $F_{1,10}=29.965$, $P<.001$). The average NREM episode duration over the entire period of SI was significantly decreased compared to baseline (from 2 min, 58 s to 56 s; $t_{(5)}=4.391$, $p=.007$). Thus, this group of rats had not only experimentally fragmented sleep, but also a reduction in total sleep time.

6 h SI, recovery at the end of the light period—Because there is a documented diurnal rhythm of BF AD levels (McKenna et al., 2003; Murillo-Rodriguez et al., 2004), we repeated the 6 h SI exposure experiment, so that the SI ended 3 h before the end of the light period, allowing the recovery period to occur in the light period. Because BF AD levels are spontaneously elevated in the dark/active period compared to the light/inactive period, an elevation in BF AD levels apparent in recovery during the light period would better support the hypothesis that this elevation reflects the homeostatic sleep drive.

During SI exposure, the percent of time awake was increased in animals exposed to SI (from 32.7% baseline to 58.3% SI exposure; $F_{1,10}=32.394$, $P<.001$). Total NREM (from 59.8% to 41.3%; $F_{1,10}=17.680$, $P=.002$) and REM (from 7.5% to 0.3%; $F_{1,10}=38.369$, $P<.001$) percentages were decreased significantly. Furthermore, the average NREM episode duration was significantly decreased (from 2 min, 13 s to 1 min, 7 s; $F_{1,10}=38.436$, $P<.001$), which again demonstrated that SI exposed rats had fragmented, not consolidated, NREM sleep. The sleep findings for this 2nd 6 h SI group were similar to those of the first 6 h SI group.

24 h SI, recovery in the later half of the light period—The number of times per hour animals were aroused from sleep (NREM or REM to wake) was significantly increased during the 24 h SI exposure. Rats during the last part of the dark period (h 13–18 of 24 h SI, 1–7AM) experienced a significant increase in number of arousals/hour compared to baseline (from 7.8 ± 2.5 baseline to 15.3 ± 4.0 SI; $F_{1,10}=16.504$, $P=.002$). This increase in number of arousals/hour continued into the last 6 h of the 24 h SI exposure, during the beginning of the light period (h 19–24 of SI, 7AM–1PM; from 11.8 ± 2.4 baseline to 21.6 ± 2.2 SI; $F_{1,10}=18.344$, $P=.002$).

As shown in Figure 1, rats began to sleep in the first hour of a 24 h exposure to SI, and the absolute amounts of NREM sleep and wakefulness resembled basal levels after the first 6 to 8 h of SI. An analysis of the total time spent in the states of sleep and wakefulness over the entire 24 h SI period revealed that the percent of time spent awake increased in the 6 animals exposed to 24 h of SI (from 49.8% baseline to 66.3% during SI; repeated measures ANOVA, $F_{1,10}=24.877$, $P=.001$), whereas NREM sleep declined (from 44.0% to 32.7%; $F_{1,10}=11.551$, $P=.007$) and REM sleep was greatly reduced (from 6.2% to 0.7%; $F_{1,10}=163.505$, $P<.001$).

during total 24 h of SI. During the SI exposure, the average duration of individual NREM episodes was limited by the onset of treadmill movement and was significantly decreased (from 2 min, 5 s to 58 s; $F_{1,10}=57.043$, $P<.001$).

Based on the inspection of the data in figure 1, the 24h SI vigilance data were subsequently analyzed in 6 h bins. The treatment X time interactions for wake ($F_{23,230}=2.236$; $P=.001$), NREM ($F_{23,230}=2.089$; $P=.003$) and REM ($F_{23,230}=2.318$; $P=.001$) percentages of the 24 h were all significant. Comparison between treatment groups revealed that, during the 24 h SI exposure, wake was elevated during hours 1–6 (second half of light period; Bonferroni corrected t-test, $t_5=-8.05$, $P<.001$) and hours 7–12 (first half of dark period; $t_5=-4.29$, $P=.008$) compared to baseline values, but not during hours 13–18 (second half of dark period) or hours 19–24 (first half of light period) of the 24 h SI exposure. Furthermore, NREM during exposure was significantly decreased during times 1–6 h ($t_5=5.917$, $P=.002$) but not 7–12 h, 13–18 h or 19–24 h. The percentage of time spent in REM sleep was significantly decreased in all time bins: 1–6 h=10.33% in control vs. 1.00% in SI ($t_5=11.626$, $P<.001$), 7–12 h=5.00% in control vs. 0.33% in SI ($t_5=3.500$, $P=.017$), 13–18 h= 3.50% in control vs. 0.67% in SI ($t_5=2.795$, $P=.038$), and 19–24 h= 5.50% in control vs. 0.17% in SI ($t_5=4.781$, $P=.005$).

When the 24 h SI exposure began at 13:00 the rats were presumably well rested since they had just experienced 6 h of undisturbed time to rest/sleep from lights on at 07:00 until SI began at 13:00. This resulted in rats having a fairly long period of wakefulness when the treadmill movement and SI began, as reported above and illustrated in figure 1. If, for the present study, the first 6 h of 24 h SI exposure was excluded from our analysis, the remaining 7–24 h of SI were not significantly different in the amount of time spent awake (from 58.2% to 60.2%) or NREM (from 38.7% to 37.8%). Thus, in the last 18h of the 24h SI exposure total NREM sleep time did not differ from baseline. In contrast, the decrease in average NREM episode duration remained in these last 18 h (from 1 min, 59 s to 1 min; $F_{1,10}=43.314$, $P<.001$); the reduction in NREM episode duration is interpreted as being caused by the SI procedure. This reduction in NREM sleep episode duration also appeared to reduce REM sleep time, at least indirectly, suggesting that long episodes of NREM sleep are needed for the rats to progress into REM sleep. Note that in the last three hours of 24 h SI (h 21–24), amounts of NREM were significantly decreased when compared to baseline levels (see Fig 1; paired samples t-test, $p=0.026$).

In summary, during SI exposure the number of arousals/hour was significantly elevated, total REM sleep time was greatly reduced, the average NREM episode duration was reduced, whereas total amounts of NREM largely stabilized as the duration of SI exposure progressed.

Effect of SI on the latency to sleep onset, a direct measure of sleepiness

6 h SI, recovery at the beginning of the dark period—The rodent MLST was performed during the recovery period (19:00–22:00; dark period) in a different group of rats ($N=8$) following this 6 h SI exposure. The average latency to sleep onset of SI exposed rats was significantly lower than the average sleep latency of the same rats before treatment (average of six trials, from 13 min, 35 s baseline to 5 min, 43 s SI; repeated measures ANOVA, $F_{1,14}=14.746$, $P=.002$). As shown in Figure 2A, this difference was most evident in the second and third trial of the rodent MSLT for SI exposures, when compared to baseline latencies (Trial 2 from 12 min, 34 s $\pm$ 3 min, 12 s baseline to 3 min, 14 s $\pm$ 1 min, 29 s SI; Trial 3 from 14 min, 34 s $\pm$ 3 min, 12 s baseline to 3 min, 51 s $\pm$ 1 min, 49 s SI). Rodent MSLT results were more variable in the first of the six trials (data not shown), presumably due to the novelty and enhanced arousal of the first gentle handling exposure. Sleep latencies in trials 4, 5, and 6, following either 6 h SI exposure, were not significantly different than baseline (matched time of day, without treatment) latencies.

6 h SI, recovery at the end of the light period—The rodent MLST was then performed during the recovery period (16:00–19:00; light period) in a different group of rats (N=8) following this 6 h SI exposure. The average sleep latency of SI exposed rats was significantly decreased compared to the average sleep latency of the same rats before treatment (average of six trials, from 7 min, 22 s baseline to 4 min, 31 s SI; $F_{1,14}=5.375$, $P=.036$). Again, the MSLT differences were most evident in the second and third trials (see Fig 2B; Trial 2 from 9 min, 6 s $\pm$ 1 min, 48 s to 2 min, 14 s $\pm$ 41 s SI; Trial 3 from 6 min, 19 s $\pm$ 1 min, 44 s baseline to 2 min, 6 s $\pm$ 42 s SI).

24 h SI, recovery in the later half of the light period—As shown in Figure 2C, the rodent MLST was performed during the recovery period (13:00–16:00; light period) following 24 h SI exposure or 24 h exercise control (Treadmill on 10 min: off 30 min, EC), and compared to baseline latency values. A significant difference between the average of sleep latency trials of the baseline, 24 hr SI, and 24 hr EC treatments was revealed ($F_{2,21}=7.323$, $P=0.004$). Furthermore, post-hoc comparisons between the three groups (Bonferroni corrected) revealed a significant difference between the baseline and 24 SI exposure (average of six trials, from 6 min, 53 s baseline to 3 min, 4 s SI; $p=0.027$) and between the 24 hr EC and 24 hr SI exposures (average of six trials, from 7 min, 52 s EC to 3 min, 4 s SI $p=0.005$). There was no significant difference between the baseline and 24 hr EC treatment. Therefore, 24h SI exposure significantly lowered the average sleep latencies compared to each rat's sleep latency under baseline conditions or following EC exposure.

NREM episode duration and NREM delta power in recovery following SI

Previous studies have demonstrated an elevation of delta power in NREM and NREM episode duration following total sleep deprivation, reflecting an elevation in the homeostatic sleep drive (e.g., Lancel and Kerkhof, 1989; Franken et al., 1991; Lancel et al., 1992). Hence, we predicted and found that levels of delta power in NREM would be elevated at the start of the recovery period following all SI exposures.

6 h SI, recovery at the beginning of the dark period—Figure 3A demonstrates a significant increase of delta power in NREM (N=6, $F_{1,10}=19.36$, $P=.001$) following 6 h of SI, when recovery was in the dark period (19:00–22:00). Average NREM episode duration (from 1 min, 32 s to 2 min, 15 s; $F_{1,10}=25.642$, $P<.001$) was significantly elevated during this recovery period.

6 h SI, recovery at the end of the light period—During this recovery period (16:00–19:00), the average delta power in NREM was significantly elevated (Figure 3B; N=6, $F_{2,20}=9.853$, $P=.012$). Furthermore, the average NREM sleep episode duration was significantly increased compared to baseline levels (from 2 min, 5 s baseline to 3 min, 20 s SI; $F_{1,10}=11.648$, $P=.007$). Regardless of the time of recovery following 6 h of SI exposure, there appears to be an elevation of the homeostatic sleep drive following 6 h of SI.

24 h SI, recovery in the later half of the light period—In addition to the 24 hr SI exposure, 4 rats were also exposed to 24 hr EC, and values of average NREM episode duration and average NREM delta power were compared to baseline. A significant difference between the average NREM episode duration of the baseline, 24 hr SI, and 24 hr EC treatments in the first 6 hr of recovery (13:00–19:00) following exposures was revealed ($F_{2,13}=9.160$, $P=0.003$). Furthermore, post-hoc comparisons between the three groups (Bonferroni corrected) revealed a significant difference between the baseline and 24 SI exposure (average of six hours, from 1 min, 56 s baseline to 3 min, 5 s SI; $p=0.006$) and between the 24 hr EC and 24 hr SI exposures (average of six hours, from 1 min, 57 s EC to 3 min, 5 s SI $p=0.015$). There was no significant difference between the baseline and 24 hr EC treatment values.

There was also a significant difference between the three treatment groups (baseline, 24 hr EC, and 24 hr SI) when comparing values of average NREM delta power in the first six hr (13:00–19:00) following exposures (Figure 3C; $N=6$; $F_{2,13}=11.249$, $P=0.001$). Post-hoc comparisons between the three groups (Bonferroni corrected) revealed a significant difference between the baseline and 24 SI exposure (average of six hours, 143% SI compared to baseline; $p=0.001$) and between the 24 hr EC and 24 hr SI exposures (average of six hours, 143% SI compared to 111% EC, where values are normalized to the average of baseline values; $p=0.028$). There was no significant difference between the baseline and 24 hr EC treatment values.

BF adenosine levels, a putative neurochemical correlate of the homeostatic sleep drive

Probe Localization—Figure 4 illustrates the location of microdialysis probe tips that were histologically confirmed to be in the targeted cholinergic BF region (black circles, $N=57$); 3 control cases (stars) from the 30 h of SI exposure group were localized outside the targeted BF region.

6 h SI, recovery at the beginning of the dark period—Figure 5A summarizes AD level fluctuation across 6 h of SI later in the light period (13:00–19:00), where the 3 h of recovery occurred in the first three h of the dark cycle (19:00–21:00). Here, there was a significant difference overall between the means of the four treatment groups [cage control, exercise control, total sleep deprivation, and SI; $F_{3,21}=12.179$, $P<.001$]. Pairwise comparisons between the treatments, using Bonferroni correction, revealed significant differences between the SI vs. cage control ($P=.044$) and exercise control ($P=.005$) groups, and total sleep deprivation vs. cage control ($P=.002$) and exercise control ($P<.001$) groups. There was not a significant difference between SI and total sleep deprivation BF AD levels. AD levels reached a zenith of 145% of baseline AD values during h 6 of SI exposure, and a zenith of 180% of baseline values during h 4 of total sleep deprivation exposure. There were no significant differences of BF AD levels between the four treatment groups in the recovery period following this 6 h of SI.

6 h SI, recovery at the end of the light period—Figure 5B summarizes AD level fluctuation across 6 h of SI earlier in the light period (10:00–16:00), where 3 h of recovery occurred at the end of the light cycle (16:00–19:00). There was a significant difference overall between the means of the three treatment groups [cage control, exercise control, and SI; $F_{2,16}=20.768$, $P<.001$]. Pairwise comparisons between the treatments, using Bonferroni correction, revealed significant differences between the SI vs. cage control ($P<.001$) and exercise control ($P<.001$). Levels of BF AD were significantly elevated, observed across 6 h of SI exposure, reaching 172% of baseline values in h 6 of SI. Furthermore, BF AD levels were significantly elevated through the 3 h of the recovery period following SI ($F_{2,16}=4.430$, $P=.029$). In summary, AD levels were significantly elevated during either treatment of 6 h of SI.

24 and 30 h of SI produced progressively elevated BF AD levels—The lack of a change in total time spent in NREM during the later part of SI exposure (h 7–24) provided an interesting opportunity to further test the hypothesis that an accumulation of AD in the BF may be a marker for, and possible mediator, of sleepiness. Figure 6 illustrates a significant difference between the means of the three treatment groups [non-BF rostral control group ($N=3$), exercise control ($N=7$), and SI ($N=8$); $F_{2,14}=8.251$, $P=.004$]. BF AD levels reached a zenith of 213% of baseline values during h 18–30 of SI exposure (light period, 07:00–19:00). Pairwise comparisons between the treatments revealed significant differences between SI treatment and both the exercise control ($P=.010$) and non-BF rostral control ($P=.023$). A significant treatment X time interaction for BF AD levels was noted ($F_{2,28}=4.434$; $P=0.007$). Subsequent post-hoc analysis of the individual data suggests that this interaction was due to significant differences between SI treatment and exercise control ($P=.031$) in the 12 h dark period (19:00–7:00, 6 to 18 h into SI), and between SI and both the exercise control ($P=.007$) and non-BF control ($P=$

021) in the 12 h light period (7:00–19:00, 18 to 30 h into SI). Thus, BF AD levels were significantly elevated during 30 h of SI, and this appeared to be a brain site-specific phenomenon, for levels did not rise in the non-BF rostral control region.

Summary of Results

Table 2 summarizes the analysis of the three SI treatment/exposure groups, evaluating our findings during SI exposure for the following measures: vigilance state changes (% Wake, % NREM, % REM), average NREM episode duration (AVE NREM), and BF AD level fluctuation (BF AD levels); and during the recovery period for the following measures: delta power in NREM (NREM delta), average NREM episode duration (AVE NREM), and average latency of sleep onset (Sleep Latency). Asterisks indicate significant differences determined in this study.

DISCUSSION

The main finding of this study was that either 6 or 24 h of SI produced an increase in measures associated with sleepiness. Specifically, during the recovery period that followed SI, rats exhibited a reduction of the average latency to sleep onset (a direct measure of sleepiness), an elevation in the average duration of NREM sleep episodes, and an elevation in NREM EEG delta power (two indirect measures of sleepiness). In addition, during the course of SI exposure, a steady elevation of BF AD was observed (a putative neurochemical correlate of sleepiness). We thus conclude that, over time periods up to 30 h, hypersomnolence stemming from our model of sleep fragmentation was evident.

Although a common experience to all of us, sleepiness can be difficult to measure quantitatively. Variations in sleepiness are considered to reflect changes in the homeostatic sleep drive, which, in turn has two main environmental determinants: 1. the duration of prior wakefulness (manipulated in the present study), and 2. the circadian time of day. In the present study sleepiness was assessed directly by the use of a rodent multiple sleep latency test. This test revealed that SI made rats sleepy. The other indirect measures of the homeostatic sleep drive supported this finding.

The SI treatment successfully modeled characteristics of the sleep profile of the OSA patient, where sleep is very fragmented, particularly limiting entry into, and the amount of, deeper stages 3–4 of NREM sleep and REM sleep (Guilleminault et al., 1976; Roehrs et al., 1985; Stepanski, 2002; Penzel et al., 2003), most likely due to the continual microarousals that end apneic moments during the OSA patient's sleep. The SI exposure effectively fragmented sleep, reducing the length of individual NREM sleep episodes from 2 min, 5 s in basal conditions to 58 s during the SI exposure. Within the first couple hours of SI exposure, rats began to sleep during the 90 s periods when the treadmill was off. Indeed, for the 24 h SI group, the total amount of NREM sleep obtained in the last 18 h of 24 h SI was equivalent to baseline values. Rats exposed to this 24 h SI model had fragmented sleep and greatly reduced amounts of REM sleep, whereas total NREM sleep time approached basal levels after the first 6 h of SI exposure. However, as noted in the results section, NREM sleep time in the last 3 hours of the 24 h SI exposure was slightly reduced compared to baseline (see Fig 1). In summary, compared to total sleep deprivation, SI exposure better models the sleep fragmentation characteristic of several clinical sleep pathologies. Indeed, the following characteristics of this SI model resemble the sleep pattern of patients with OSA: the frequency of arousals (~30 arousals/hr), the large reduction of REM sleep time, the restriction of average NREM sleep episode duration, and the feature that 24h total amounts of NREM sleep time approach baseline levels.

The increase in the average duration of NREM sleep episodes and NREM EEG delta power during the recovery period after SI exposure supports our hypothesis that the homeostatic sleep

drive was elevated by the SI treatment. Various laboratories have proposed that delta power of NREM reflects the homeostatic sleep drive following such sleep manipulations as total sleep deprivation (Borbely and Neuhaus, 1979; Borbely, 1982; Franken et al., 1991). Furthermore, an increase in the average NREM episode duration in the recovery period following sleep deprivation is also accepted as indicative of an increase in sleep pressure (Lancel and Kerhof, 1989; Franken et al., 1991; Lancel et al., 1992).

Although the measurement of sleep onset latencies using the rodent MSLT test provides a direct measure of sleepiness (i.e., sleep propensity), it may not be as sensitive a measure as are polysomnographic indicators of sleepiness. Thus, a decreased sleep onset latency was observed in the first two hours following 24 h SI whereas by the third hour of the recovery period the sleep latencies approached baseline values. In contrast, average NREM EEG delta power in the recovery period was elevated for more than three hours suggesting this may be a more sensitive, albeit indirect, measure of sleepiness.

Recently, Polotsky et al. (2006) exposed C57BL/6J mice to experimental sleep fragmentation for 12 h/day in the light period and found no evidence of increased NREM delta power (analyzed in 12 h bins), or other changes in sleep that would suggest an increased sleep drive. However, in the present study, rats were on the SI schedule continuously for 24 h, a design that prevents the rats from napping during the dark period to make up for sleep lost during the light/inactive period. The present findings also indicate that increases in NREM delta power were evident in the first hours of the recovery period after SI.

Despite two days of habituation to the treadmill apparatus, during the first 6 h of SI exposure total sleep time was significantly reduced. Hence, one 6 h SI group was compared to rats exposed to 6h of total sleep deprivation produced by gentle handling (i.e., a positive control group). The elevations of BF AD levels produced by 6 h of SI and 6 h of total sleep deprivation were very similar.

Both short term (6 h) and longer term (30 h) SI exposures lead to significantly elevated levels of AD in BF. For example, 6 h of SI produced ~150% elevation of BF AD levels, very similar to the increase that is seen following 6 h of total sleep deprivation in the cat or rat (Porkka-Heiskanen et al., 1997, 2000; Basheer et al., 1999; Kalinchuk et al., 2003; McKenna et al., 2003; Murillo-Rodriguez et al., 2004). Even though NREM sleep time approached baseline (control matched time of day) values in the last 18 h of the 24 h SI treatment (Figure 1) BF AD levels continued to rise, suggesting that sleep fragmentation alone (defined herein as the decrease in the average NREM episode duration), as opposed to cumulative prior wakefulness, appears to produce an increase in BF AD.

A growing body of evidence supports the role of AD as a mediator of the sleepiness following prolonged wakefulness (i.e., AD is an endogenous somnogen), including the findings of this study. The SI-induced elevation of BF AD levels appeared to be specifically due to the sleep manipulation and not to locomotor activity, since BF AD was not elevated in the exercise control rats. Also, SI did not elevate AD levels when microdialysis probes were placed just anterior to our target site, supporting the previous finding that the elevation of AD in response to sleep loss is brain site specific (Porkka-Heiskanen et al., 2000).

Conclusion

The data indicate that either 6 or 24 h of SI can elevate behavioral and electrographic measures of sleepiness. The SI-induced increase in the homeostatic sleep drive is also correlated with an elevation of BF extracellular AD levels, a proposed neurochemical mediator of sleepiness. The combined findings are consistent with the following model: similar to total sleep deprivation, sleep fragmentation leads to an increase of AD in the BF which inhibits the activity of wake-

promoting BF neurons, leading to decreased cortical activation and a subsequent increase in sleepiness.

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References

- Alam MN, Szymusiak R, Gong H, King J, McGinty D. Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. *J Physiol* 1999;521:679–90. [PubMed: 10601498]
- Basheer R, Porkka-Heiskanen T, Stenberg D, McCarley RW. Adenosine and behavioral state control: adenosine increases c-Fos protein and AP1 binding in basal forebrain of rats. *Mol Brain Res* 1999;73:1–10. [PubMed: 10581392]
- Basheer R, Strecker RE, Thakkar MM, McCarley RW. Adenosine and sleep wake regulation. *Prog Neurobiol* 2004;73:379–96. [PubMed: 15313333]
- Benington JH, Heller HC. Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol* 1995;45:347–60. [PubMed: 7624482]
- Benington JH, Kodali SK, Heller HC. Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. *Brain Res* 1995;692:79–85. [PubMed: 8548323]
- Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR, Shiromani PJ. Adenosine and sleep homeostasis in the basal forebrain. *J Neurosci* 2006;26:8092–8100. [PubMed: 16885223]
- Bonnet MH. Sleep restoration as a function of periodic awakening, movement, or electroencephalographic change. *Sleep* 1987;10:364–373. [PubMed: 3659734]
- Borbely, AA. Sleep regulation: circadian rhythms and homeostasis. In: Ganten, D.; Pfaff, editors. *Current Topics in Neuroendocrinology*. Germany: Springer-Verlag, Berlin, Heidelberg; 1982. p. 83-104.
- Borbely AA, Neuhaus HU. Sleep-deprivation: effects on sleep and EEG in the rat. *J Comp Physiol* 1979;133:71–87.
- Coleman RM, Roffwarg HP, Kennedy SJ, Guilleminault C, Cinque J, Cohn MA, Karacan I, Kupfer DJ, Lemmi H, Miles LE, Orr WC, Phillips ER, Roth T, Sassin JF, Schmidt HS, Weitzman ED, Dement WC. Sleep-wake disorders based on a polysomnographic diagnosis. A national cooperative study. *JAMA* 1982;247:997–1003. [PubMed: 7057593]
- Day R, Gerhardstein R, Lumley A, Roth T, Rosenthal L. The behavioral morbidity of obstructive sleep apnea. *Prog Cardiovasc Dis* 1999;41:341–54. [PubMed: 10406328]
- Dunwiddie TV, Worth T. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. *J Pharmacol Exp Ther* 1982;220:70–6. [PubMed: 7053424]
- Franken P, Dijk DJ, Tobler I, Borbely AA. Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. *Am J Physiol* 1991;261:198–208.
- Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999;51:83–133. [PubMed: 10049999]
- Fulga I, Stone TW. Comparison of an adenosine A1 receptor agonist and antagonist on the rat EEG. *Neurosci Lett* 1998;244:55–9. [PubMed: 9578143]
- Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. *Annu Rev Med* 1976;27:465–84. [PubMed: 180875]
- Hoyt BD. Sleep in patients with neurologic and psychiatric disorders. *Prim Care* 2005;32:535–48. [PubMed: 15935199]
- Kalinchuk AV, Urrila AS, Alanko L, Heiskanen S, Wigren HK, Suomela M, Stenberg D, Porkka-Heiskanen T. Local energy depletion in the basal forebrain increases sleep. *Eur J Neurosci* 2003;17:863–9. [PubMed: 12603276]

- Kryger, MH.; Roth, T.; Dement, WC. Principles and Practice of Sleep Medicine. 3. Philadelphia, Pennsylvania: W.B. Saunders Company; 2000.
- Lancel M, Kerkhof GA. Effects of repeated sleep deprivation in the dark- or light-period on sleep in rats. *Physiol Behav* 1989;45:289–97. [PubMed: 2756014]
- Lancel M, van Riezen H, Glatt A. Enhanced slow-wave activity within NREM sleep in the cortical and subcortical EEG of the cat after sleep deprivation. *Sleep* 1992;15:102–18. [PubMed: 1579784]
- McKenna JT, Dauphin LJ, Mulkern KJ, Stronge AM, McCarley RW, Strecker RE. Nocturnal elevation of extracellular adenosine in the rat basal forebrain. *Sleep Research Online* 2003;5:155–160.
- Mendelson WB. Sleep-inducing effects of adenosine microinjections into the medial preoptic area are blocked by flumazenil. *Brain Res* 2000;852:479–81. [PubMed: 10678777]
- Murillo-Rodriguez E, Blanco-Centurion C, Gerashchenko D, Salin-Pascual RJ, Shiromani PJ. The diurnal rhythm of adenosine levels in the basal forebrain of young and old rats. *Neuroscience* 2004;123:361–70. [PubMed: 14698744]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1998.
- Penzel T, Kantelhardt JW, Lo C, Voigt K, Vogelmeier. Dynamics of heart rate and sleep stages in normals and patients with sleep apnea. *Neuropsychopharmacol* 2003;28:S48–S53.
- Polotsky VY, Rubin AE, Balbir A, Dean T, Smith PL, Schwartz AR, O'Donnell CP. Intermittent hypoxia causes REM sleep deficits and decreases EEG delta power in NREM sleep in the C57BL/6J mouse. *Sleep Med* 2006;7:7–16. [PubMed: 16309961]
- Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW. Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 1997;276:1265–8. [PubMed: 9157887]
- Porkka-Heiskanen T, Strecker RE, McCarley RW. Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep; an in vivo microdialysis study. *Neuroscience* 2000;99:507–17. [PubMed: 11029542]
- Portas CM, Thakkar M, Rainnie DG, Greene RW, McCarley RW. Role of adenosine in behavioral state modulation: a microdialysis study in the freely moving cat. *Neuroscience* 1997;79:225–35. [PubMed: 9178878]
- Radulovacki M. Role of adenosine in sleep in rats. *Rev Clin Basic Pharmacol* 1985;5:327–39.
- Roehrs T, Conway W, Wittig R, Zorick F, Sichelsteel J, Roth T. Sleep-wake complaints in patients with sleep-related respiratory disturbances. *Am Rev Respir Dis* 1985;132:520–523. [PubMed: 4037527]
- Roth T, Roehrs T, Rosenthal L. Hypersomnolence and neurocognitive performance in sleep apnea. *Curr Opin Pulm Med* 1995;1:488–90. [PubMed: 9363087]
- Roth T, Roehrs TA. Etiologies and sequelae of excessive daytime sleepiness. *Clin Ther* 1996;18:562–76. [PubMed: 8879887]
- Sateia MJ, Greenough G, Nowell P. Sleep in neuropsychiatric disorders. *Semin Clin Neuropsychiatry* 2000;5:227–37. [PubMed: 11291019]
- Shiromani PJ, Velazquez-Moctezuma J, Overstreet D, Shalauta M, Lucero S, Floyd C. Effects of sleep deprivation on sleepiness and increased REM sleep in rats selectively bred for cholinergic hyperactivity. *Sleep* 1991;14:116–120. [PubMed: 1650958]
- Soldatos CR, Paparrigopoulos TJ. Sleep physiology and pathology: pertinence to psychiatry. *Int Rev Psychiatry* 2005;17:213–28. [PubMed: 16194793]
- Stepanski EJ. The effect of sleep fragmentation on daytime function. *Sleep* 2002;25:268–76. [PubMed: 12003157]
- Steriade, M.; McCarley, RW. Brain control of wakefulness and sleep. New York: Kluwer Academic/Plenum, New York; 2005.
- Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, Rainnie DG, Portas CM, Greene RW, McCarley RW. Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav Brain Res* 2000;115:183–204. [PubMed: 11000420]
- Szymusiak R, Alam N, McGinty D. Discharge patterns of neurons in cholinergic regions of the basal forebrain during waking and sleep. *Behav Brain Res* 2000;115:171–82. [PubMed: 11000419]

- Thakkar MM, Delgiacco RA, Strecker RE, McCarley RW. Adenosinergic inhibition of basal forebrain wakefulness - active neurons: A simultaneous unit recording and microdialysis study in freely behaving cats. *J Neurosci* 2003a;122:1107–13.
- Thakkar MM, Winston S, McCarley RW. A1 receptor and adenosinergic regulation of sleep-wakefulness: effects of antisense to the A1 receptor in the cholinergic basal forebrain. *J Neurosci* 2003b;23:4278–87. [PubMed: 12764116]
- Ticho SR, Radulovacki M. Role of adenosine in sleep and temperature regulation in the preoptic area of rats. *Pharmacol Biochem Behav* 1991;40:33–40. [PubMed: 1780343]
- Veasey SC, Yeou-Jey H, Thayer P, Fenik P. Murine multiple sleep latency test: phenotyping sleep propensity in mice. *Sleep* 2004;27:388–93. [PubMed: 15164889]
- Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;328:1230–5. [PubMed: 8464434]

LIST OF ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AD	adenosine
ANOVA	analysis of variance
AP	anterior-posterior
BF	basal forebrain
CC	cage control group
cm	centimeter
EC	exercise control group
EEG	electroencephalograph
EMG	electromyography
FEP	a polymer of tetrafluoroethylene and hexafluoropropylene
HPLC	high performance liquid chromatography
H	horizontal
h	hour(s)
Hz	

	hertz
i.m	intramuscular
i.p	intraperitoneal
L	lateral
m	meter
m/s	meter per second
min	minute(s)
MSLT	multiple sleep latency test
NREM	non-rapid eye movement sleep
REM	rapid eye movement sleep
s	second(s)
S.D	standard deviation
SI	sleep interruption
μl	microliter
UV	ultraviolet
W	wakefulness

Figure 1.

The Sleep interruption paradigm (SI) increased the amount of time spent awake during the first 6 h of exposure, but this difference largely disappears during the final 18 h of SI. Illustrated here are the hourly percentages of wakefulness (top panel), NREM (middle panel), and REM (bottom panel) during 24 h of SI exposure (SI, N=6, 13:00–12:00), beginning at 13:00 (e.g., time bin 13 on the X axis refers to the recording taken for 13:00–14:00) and ending at 13:00 (12 recording for 12:00–13:00 of the next day). Light/dark cycle is 07:00/19:00. Open circles represent the mean percentages for the baseline day, and closed black circles represent hourly means of vigilance states during the 24 h of SI-exposure. Baseline values were of the same animals, taken from the day preceding exposures; that is, each animal was used as its own

control. * indicates significant difference between 6 h binned data of baseline and wake amounts of SI-exposed animals, $p < 0.05$, paired samples t-test with Bonferroni correction. Light/dark bars below the graphic indicate the light (largely inactive) and dark (largely active) periods of the animal.

Figure 2.

The rodent multiple sleep latency test (MSLT) demonstrated a significant decrease in the latency to sleep onset in the recovery period following each of the three sleep interruption (SI) exposures. Light/dark cycle was 7:00/19:00. Average sleep latencies were decreased in the second and third trials, regardless of the time of recovery after SI (in the dark (Panel A) or light (Panel B) period). In panels A and B, open bars represent average baseline values of sleep latencies (N=8 per SI exposure), and solid bars represent the average values of sleep latencies following 6 h SI, for the second (after .5 h of recovery sleep) and third (after 1 h of recovery sleep) rodent MSLT trials, with recovery at the beginning of the dark period (Panel A, N=8); or 6 h SI, with recovery at the end of the light period (Panel B, N=8). In Panel C, all six trials over the 3 h of the rodent MSLT are depicted for 24 h SI treatment, with recovery in the middle of the light period (N=8). Latency values following 24 h SI were significantly decreased (black circles) when compared to either baseline (open circles) or exercise control (black triangles). The data plotted are means $\pm$ SEM.

Figure 3.

NREM delta power was elevated during the recovery period that followed 6 or 24h of SI, indicating an elevated homeostatic sleep drive. Data are Fast Fourier Transform analyses of hourly delta power in NREM during recovery after SI (black circles), baseline (open circles), or 24 h exercise control (black triangles). Light/dark cycle was 07:00/19:00. In Panel A, the recovery period is at the beginning of the dark period (N=6, 19:00–22:00) following 6 h SI (13:00–19:00). In Panel B, the recovery period is in the last 3 h of the light period (N=6, 16:00–19:00) following 6 h SI (10:00–16:00). Average NREM delta power was increased in the recovery period following 6 h SI, regardless of when recovery occurred (in the dark (Panel A) or light (Panel B) period). Lastly, in Panel C, the recovery period is the last 6 h of the light period (N=6, 13:00–19:00) following 24 h SI exposure (13:00–13:00). Each animal was used as its own control, and baseline values were those of the day preceding the SI exposures.

Figure 4.

Schematic representation of the ventral forebrain region (3 coronal levels at -2.0, -0.26 and -0.40 mm caudal of bregma) illustrating the histologically identified locations of the ventral tip of 57 probes (black circles). Stars indicate 3 probe sites that were outside our target area of the cholinergic basal forebrain region (BF; 0.20 mm rostral to bregma). Figure adapted from Paxinos and Watson (1998). Scale bar = 1 mm. Abbreviations: ac, anterior commissure; Acb, nucleus accumbens; acp, anterior commissure, posterior part; CPu, caudate putamen; HDB, nucleus of the horizontal limb of the diagonal band; LPO, lateral preoptic area; MCPO, magnocellular preoptic nucleus; SI, substantia innominata; VP, ventral pallidum.

Figure 5.

Irrespective of whether 6 h of sleep interruption (SI) was terminated at the end of the light period (panel A), or during the light period (panel B), extracellular basal forebrain (BF) adenosine (AD) levels were significantly elevated during SI treatment compared to control groups. (A) BF AD levels are significantly elevated during SI exposure (black circles, N=7) compared to either the exercise (10 min treadmill on: 30 min treadmill off, black triangles, N=7) or cage control groups (open triangles, N=4). BF AD levels during SI treatment are not significantly different than levels during total sleep deprivation produced by gentle handling (open squares, N=6, data modified from McKenna et al., 2003). BF AD levels during SI were highest at h 6 of SI (145%), and SD at h4 (180%). Light/dark cycle is 07:00/19:00. 1 h samples

were collected across the assay time, and the data plotted are means $\pm$ SEM expressed as the % of baseline. (B) BF AD levels during SI exposure from 10:00–16:00 (N=7) were significantly elevated compared to either the exercise (N=7) or cage control groups (N=4). The highest elevation of BF AD levels during SI exposure was at h 6 of SI (172%).

Figure 6.

Extracellular BF AD levels rise were significantly elevated during 30 h of sleep interruption (SI, N=8, black circles) compared to exercise controls (N=7, open triangles) or in animals with non-basal forebrain collection sites (N=3; black triangles). Light/dark cycle is 07:00/19:00. BF AD levels reached approximately 210% of baseline AD levels by the light period (07:00–19:00) of the second day after the treadmill SI onset (18 to 30h after treadmill onset). 1 h samples were collected across the assay time, and the data plotted are means±SEM expressed as the % of baseline samples (07:00–13:00). * indicates significant difference between exercise controls and BF AD levels in SI-exposed animals ($p<0.05$, paired samples t-test, with Bonferroni correction). [*] indicated significant differences between BF AD levels in SI-exposed animals compared to BF AD levels in both exercise control and non-basal forebrain control groups ($p<0.05$, paired samples t-test, with Bonferroni correction).

Table 1**Summary of Experimental Groups**

Table 1 for McKenna et al., "Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness", Submitted Sept. 18, 2006 to Neuroscience

	Light/Dark Schedule	Time of Exposure	Length of Exposure	Time of Recovery
6 h SI, rec dark	7AM/7PM	1–7PM	6 h	7–10PM
6 h SI, rec light	7AM/7PM	10AM–4PM	6 h	4–7PM
24–30 h SI	7AM/7PM	1PM–1PM	24–30 h	1–7PM

Summary of Results

Table 2 for McKenna et al., “Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness”, Submitted Sept. 18, 2006 to Neuroscience

Table 2

	During SI Exposure				BF AD levels
	% Wake	% NREM	% REM	Ave NREM	
6 h SI, rec dark	29.2%BL to 65.0%*	62.0%BL to 34.7%SI*	8.8%BL to 0.2%SI*	178s BL to 56s SI*	Increase*
6 h SI, rec light	32.7%BL to 58.3%*	59.8%BL to 41.3%SI*	7.5%BL to 0.3%SI*	133s BL to 67s SI*	Increase*
24-30 hr SI	49.8%BL to 66.3%*	44.0%BL to 32.7%SI*	6.2%BL to 0.7%SI*	125s BL to 58s SI*	Increase*
During the Recovery Period					
6 h SI, rec dark	NREM delta		Ave NREM		Sleep Latency
	Increase*		92s BL to 135s SI*		
	Increase*		125s BL to 200s SI*		
	Increase*		125s BL to 199s SI*		
6 h SI, rec light					13:35m BL to 5:43m SI*
24-30 hr SI					7:22m BL to 4:31m SI*
					6:53m BL to 3:04m SI*